UNIVERSITY OF KWAZULU-NATAL

THE EXTRACTIVES FROM *SOPHORA VELUTINA* AND *CALPURNIA AUREA* AND THEIR BIOLOGICAL ACTIVITY

2012

ERICK KIPKOECH KORIR

THE EXTRACTIVES FROM *SOPHORA VELUTINA* AND *CALPURNIA AUREA* AND THEIR BIOLOGICAL ACTIVITY

ERICK KIPKOECH KORIR

2012

A thesis submitted to the school of Chemistry, Faculty of Science and Agriculture, University of KwaZulu-Natal, Westville, for the degree of Doctor of Philosophy.

This Thesis has been prepared according to **Format 4** as outlined in the guidelines from the Faculty of Science and Agriculture which states:

This is a thesis in which chapters are written as a set of discrete research papers, with an overall introduction and final discussion. Where one (or all) of the chapters have already been published. Typically these chapters will have been published in internationally recognized, peer- reviewed journals.

As the candidate's supervisor, I have approved this thesis for submission.

Supervisor:

Signed: ----------Name: ---------- Date: ----------

Abstract

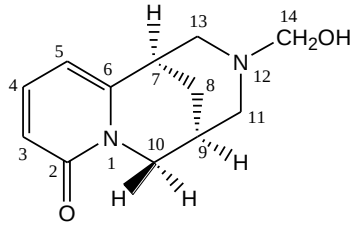
This work is an account of the phytochemistry and biological activity of two related plant species within the plant family the Fabaceae, *Sophora velutina* from the subtribe Sophoreae and *Calpurnia aurea* of the subtribe Podalyrieae. Members of this family are known to contain quinolizidine alkaloids and flavonoids, which are chemotaxonomic markers in the Fabaceae.

The phytochemical investigation of *Sophora velutina* resulted in the isolation of ten compounds, including five novel quinolizidine alkaloids, *N*-methylenehydroxycytisine (**A-1**), 7-hydroxylupanine (**A-2**), 6,7-dihydroxylupanine (**A-3**) and 17-oxo-thermopsine (**A-4**) from the fruits and velutinine (**A-5**) from the bark along with the known quinolizidine alkaloids *N*-methylcytisine (**A-6**) and cytisine (**A-7**), and a cinnamate ester, methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate (**A-8**) and triterpenoids lup-20(29)-ene-3β-ol (**A-9**) and 12-oleanen-3-one (**A-10**). The isolated compounds were tested for their antibacterial activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa*. *P. aeruginosa* showed resistance against eight of the ten samples tested with only the cinnamate ester and the steroid 12-oleanen-3-one (**A-10**) being slightly active at 200 and 175 μg mL^{-1}, respectively. However, the quinolizidine alkaloid, *N*-methylcytisine (**A-6**) and 12-oleanen-3-one (**A-10**) showed good antibacterial activity against *E. faecalis*, with MIC values of 20.8 and 10.9 μg mL^{-1}, respectively, with 17-oxo-thermopsine (**A-4**), another quinolizidine alkaloid, and the cinnamate ester showing moderate antibacterial activity against *E. faecalis* at concentrations of 125 μg mL^{-1} and 100 μg mL^{-1}, respectively.

Calpurnia aurea yielded five isoflavones, 4′,5,7-trihydroxyisoflavone (**B-1**), 7,3′-dihydroxy-5′-methoxyisoflavone (**B-2**), 7-hydroxy-4′,8-dimethoxyisoflavone (**B-3**), 7-acetoxy-4′,8-dimethoxyisoflavone (**B-4**) and 3',7-dihydroxy-4′,8-dimethoxyisoflavone (**B-5**), a pterocarpan (3-acetoxy-9-methoxypterocarpan) (**B-6**) and a quinolizidine alkaloid (calpurnine) (**B-7**) all of which were isolated from the stem and bark. These isoflavones were screened for *in vitro* anticancer activity against breast (MCF7), renal (TK10) and melanoma (UACC62) human cell lines, where 3',7-dihydroxy-4′,8-dimethoxyisoflavone (**B-5**) was found to be the most active amongst all the compounds tested, followed by 3',7-dihydroxy-5'-methoxyisoflavone (**B-2**), also with a hydroxyl and methoxy group on the phenyl ring but in the 3' and 5' positions, respectively.

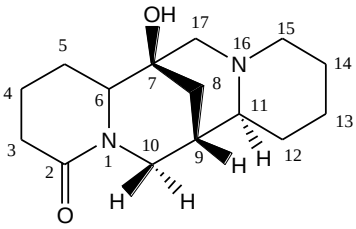
Elucidation of the compounds was done mainly by 1D and 2D NMR spectroscopy together with mass spectrometry, infrared, and ultraviolet spectroscopy. Antibacterial and anticancer assays were carried out using standard assays at the Centre for Scientific and Industrial Research (CSIR), Pretoria, South Africa.

Compounds isolated from *Sophora velutina* subsp. *zimbabweensis*



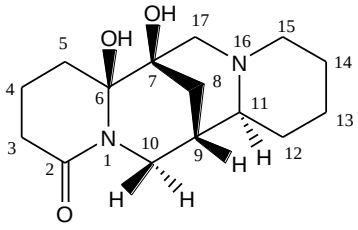
A-1

$C_{12}H_{16}N_2O_2$
Exact Mass: 220.1212



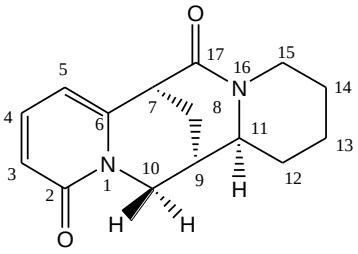
A-2

$C_{15}H_{24}N_2O_2$
Exact Mass: 264.1838



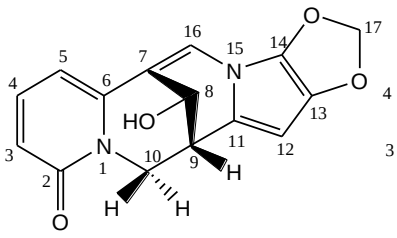
A-3

$C_{15}H_{24}N_2O_3$
Exact Mass: 280.1787



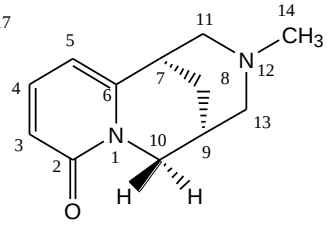
A-4

$C_{15}H_{18}N_2O_2$
Exact Mass: 258.1368



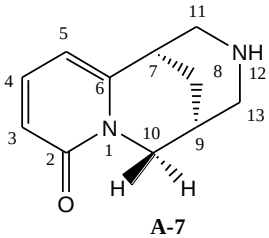
A-5

$C_{15}H_{12}N_2O_4$
Exact Mass: 284.0797



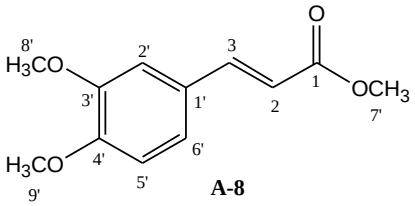
A-6

$C_{12}H_{16}N_2O$
Exact Mass: 204.1263



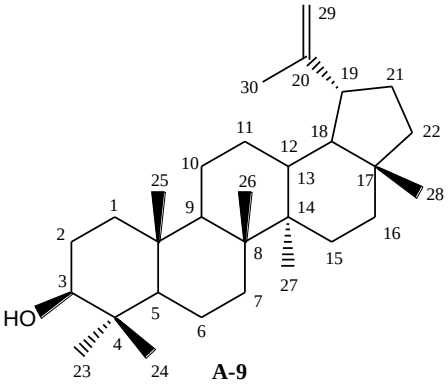
A-7

$C_{11}H_{14}N_2O$
Exact Mass: 190.1106



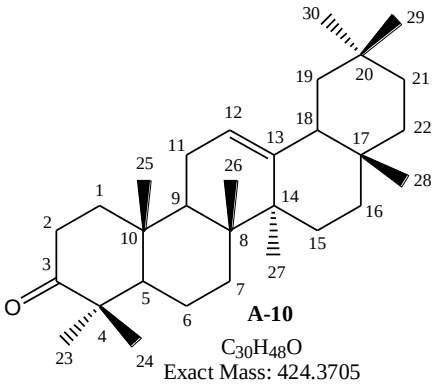
A-8

$C_{12}H_{14}O_4$
Exact Mass: 222.0892



A-9

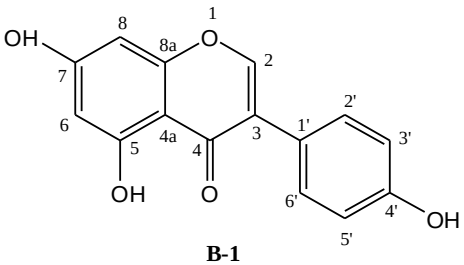
$C_{30}H_{50}O$
Exact Mass: 426.3862



A-10

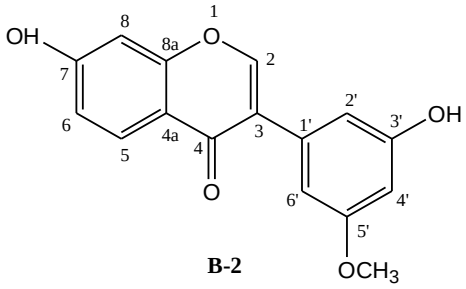
$C_{30}H_{48}O$
Exact Mass: 424.3705

Compounds isolated from *Calpurnia aurea*



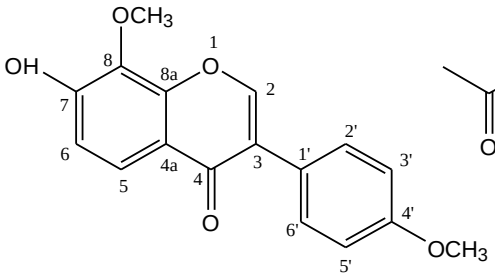
B-1

$C_{15}H_{10}O_5$
Exact Mass: 270.0528



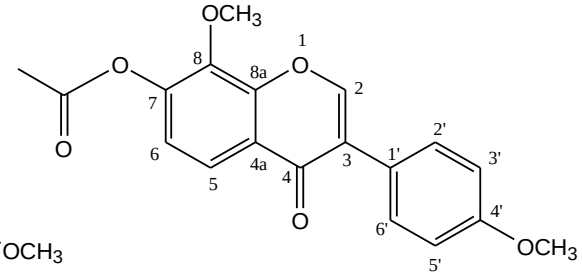
B-2

$C_{16}H_{12}O_5$
Exact Mass: 284.0685



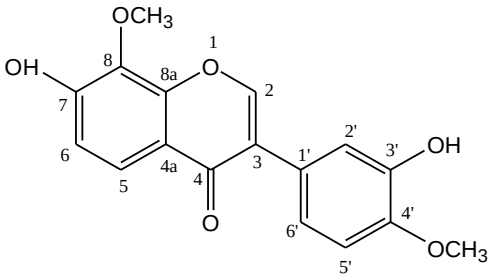
B-3

$C_{17}H_{14}O_5$
Exact Mass: 298.0841



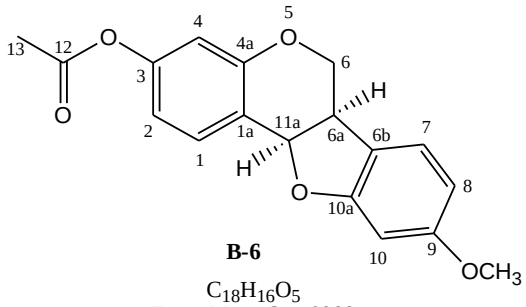
B-4

$C_{19}H_{16}O_6$
Exact Mass: 340.0947



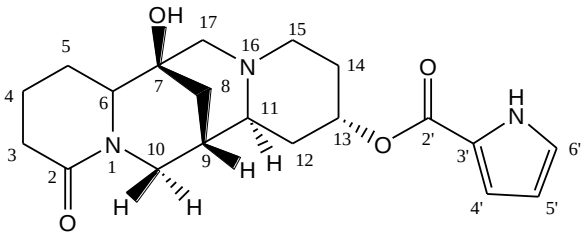
B-5

$C_{17}H_{14}O_6$
Exact Mass: 314.0790



B-6

$C_{18}H_{16}O_5$
Exact Mass: 312.0998



B-7

$C_{20}H_{27}N_3O_4$
Exact Mass: 373.2002

ABBREVIATIONS

^{13}C NMR	C-13 nuclear magnetic resonance spectroscopy
^{1}H NMR	proton nuclear magnetic resonance spectroscopy
Ac	acetate
aq	aqueous
aq EtOH	aqueous ethanol
aq MeOH	aqueous methanol
br	broad resonance
c	concentration
cc	column chromatography
cGMP	cyclic guanosine monophosphate
CO	*Cladosporium oxysporum*
CD_3OD	deuterated methanol
$CDCl_3$	deuterated chloroform
COSY	correlated spectroscopy
COX	cyclooxygenase
CSIR	Council for Scientific and Industrial Research
d	doublet
dd	double doublet
DEPT	distortionless enhancement by polarization transfer
DNA	deoxyribonucleic acid
DNP	dictionary of natural products
EIMS	electron impact mass spectroscopy
FO	*Fusarium oxysporum*
GI	growth inhibition
HMBC	heteronuclear multiple bond coherence
HPLC	high pressure liquid chromatography
HREIMS	high resolution electron impact mass spectroscopy
HSQC	heteronuclear single quantum coherence
IR	infrared
m	multiplet

MB	*Marssonina brunnee*
Me	methyl
MIC	minimum inhibitory concentrations
Mp	melting point
MS	mass spectroscopy
NOESY	nuclear overhauser effect spectroscopy
PDE-5	Phosphodiesterase type 5
RSA	radical scavenging activity
s	singlet
SRB	sulforhodamine
SS	*Sphaeropsis sapinia*
t	triplet
TCA	trichloroacetic acid
TGI	total growth inhibition
TLC	thin layer chromatography
UV	ultraviolet
VP	*Valsa pini*
MIC	minimum inhibitory concentration

DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, Erick Kipkoech Korir declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written but the general information attributed to them have been referenced
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed ……

DECLARATION 2-PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1

Korir, E., Kiplimo, J.J., Crouch, N., Moodley, N., Koorbanally, N.A. **2012**. Quinolizidine Alkaloids from *Sophora velutina* subsp. *zimbabweensis* (Fabaceae: Sophoreae), *Natural Products Communications*, **7** (8), 999-1003.

Publication 2

Korir, E., Kiplimo, J.J., Crouch, N., Moodley, N., Koorbanally, N.A. **2012**. 7-Hydroxylupanine and 17-oxo-thermopsine from *Sophora velutina* subsp. *zimbabweensis*, submitted to *Natural Products Communications*.

Publication 3

Korir, E., Kiplimo, J.J., Crouch, N., Moodley, N., Koorbanally, N.A. Isoflavones from *Calpurnia aurea* Subsp. *aurea* and their anticancer activity, manuscript submitted to *Journal of Medicinal Plants Research*.

From all the above publications, my role included carrying out all the experimental work and contributing to the writing of the publications along with my supervisor. The other co-authors contribution was that of an editorial nature and checking on the scientific content and my correct interpretation. Based on their expertise, they have added minor parts to the manuscripts.

Signed: ……..

ACKNOWLEDGEMENTS

I am greatly indepted to my mentor and supervisor Dr Neil Anthony Koorbanally for first accepting me into his great research group- Natural Products Research Group (NPRG) but also for facilitating a smooth running of my studies with his grant holders bursary. His kindness, humility and calmness provided a good learning and research environment and also motivated me to work harder day by day. To me you are special.

Appreciation also goes out to Dr Nivan Moodley for making it possible for me to run the biossays and HREIMS at the CSIR and to Prof. Neil Crouch and Dr R. Clark of Pretoria for facilitating access to plant material for research.

To my colleagues and members of the Natural Products Research Group, Dr Chantal Koorbanally, Dr Joseph Magadula (JJ), Dr Abdelhafeez Mohamed, Jil, Vusi Mchunu, Maya Makatini, Gugulethu Ndlovu, Dr Elizabeth Mwangi, Shiksha Dukhea, Ibrahim Hamisu, Edith Sebata, Damien Tshibangu and Kaalin Gopaul, you have made my stay memorable and I learned a lot by interacting with you in the lab. Many thanks go to Chantal, JJ, Abdel, Phil and Dulcie for giving me the best reception in the lab and guiding me through the best research practices. I will remember this forever.

I am also grateful to Dilip for inducting me to the NMR instrument and Pret Parel and Anita Naidoo for inducting me to the MS, UV and IR instruments. I am indeed grateful to the entire staff in the School of Chemistry for providing a good learning environment and making me feel at home.

I will not forget my Kenyan brothers in the School of chemistry, Waudo, Changamu, Kibe, Maingi, Musyoka, Mwangi and the rest as well as Isaac (Newlight international) for the good company away from home. I am also grateful to Cheplogoi, Langat and Rotich for being my best ambassadors and role models. To you I say Kongoi Nebo Sobon.

My deepest gratitude goes to my wife Beatrice. You are a gift to me from God and thanks for your kindness and humility. You have always been extremely supportive and patient with me

apart from the many sacrifices you have made for the family. This is not in vain and God Bless you. To my children Kipngetich, Chepkemoi, Kiptoo and Kipngeno, thank you so much for your encouragement, best wishes and prayers. To my parents, thank you for your encouragement in all these years.

Most importantly, if it was not for your grace and favours Lord, I would not have made it this far. Thank you.

I am grateful to the National Research Foundation (NRF) of South Africa through the Thuthuka Programme for financial support and a grant holder's bursary.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

Chapter 1. Introduction to the Fabaceae

1.1 Phylogeny

The Fabaceae is a member of the Angiospermae (flowering plants) and consists of about 750 genera with over 18,000 species (ILDIS, 2001).

The plants which are mainly trees, shrubs or climbers are grouped into the three subfamilies (Scheme 1) based on morphological characteristics using either the appearance of their flowers, pods or leaves. These genera are distributed between three subfamilies, the Papilionoideae, Mimosoideae and Caesalpinoideae.

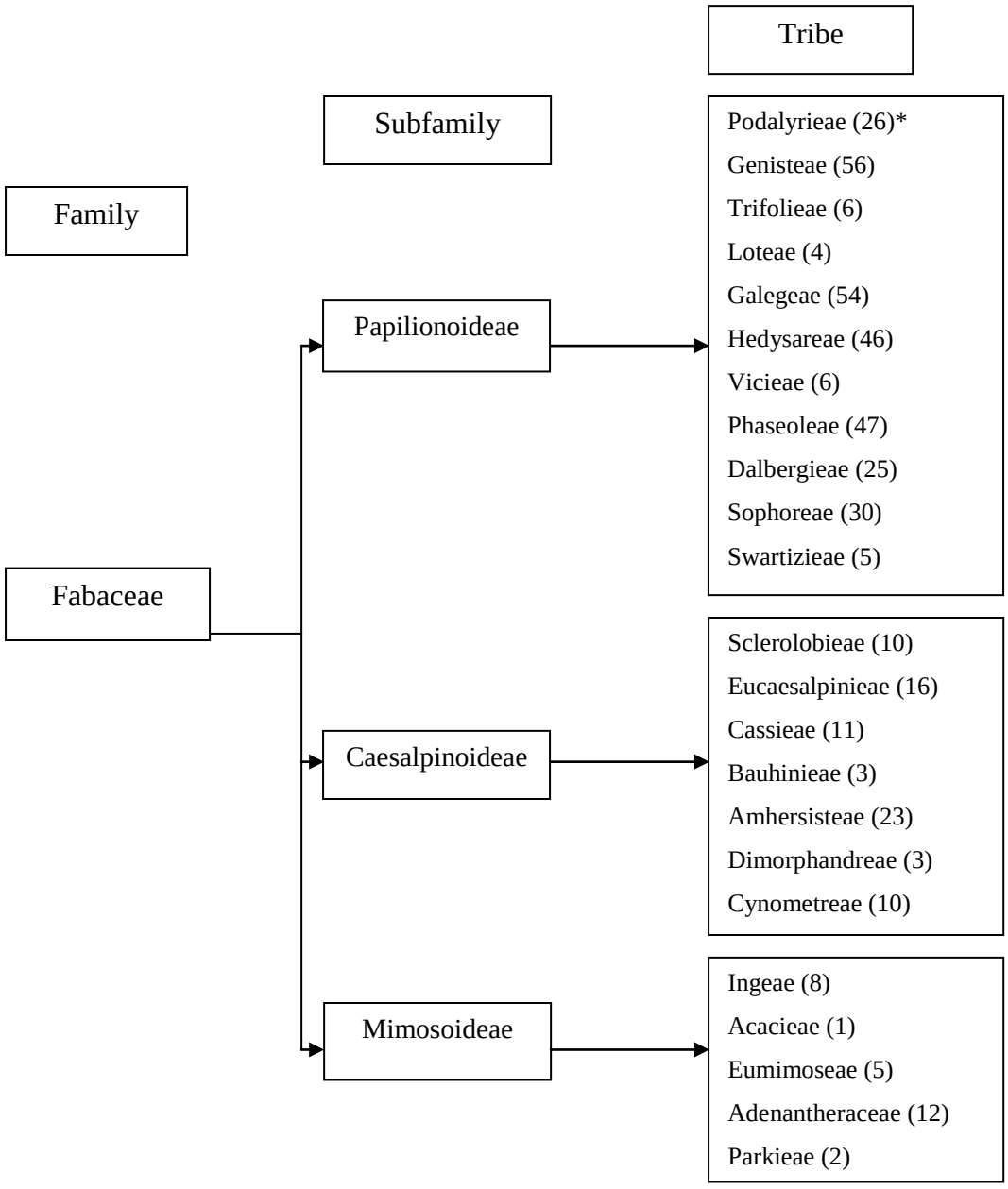
In the Mimosoideae, the flowers within a whorl are radially symmetric with similar petals in shape and size and their leaves are bipinnate (having leaflets on each side of a common axis, which are further subdivided into smaller leaflets) and the flowers of the Caesalpinoideae and Papilionoideae are bilaterally symmetric with the size and the shape of the petals in a given whorl being different. The differences between the Caesalpinioideae and Papilionoideae are twofold: the sepals which are often separate in Caesalpinioideae are united at the base in the Papilionoideae and the radicles in the seeds are straight in Caesalpinioideae and curved in Papilionoideae (Germishuizen, 2000).

The species studied in this work belong to two tribes in the Papilionoideae subfamily, the Podalyrieae and Sophareae. Initially, the two plants were considered to belong to the same tribe Sophoreae but later studies have placed *Calpurnia* (Polhill, 1994) together with *Cadia* (van Wyk

and Shuttle, 1995) under the tribe Podalyrieae. The two tribes differ in that *Sophoreae* are trees, shrubs and climbers having pinnate leaves while Podalyrieae are mainly shrubs with palmate or digitative leaves (emanating from a point or a centre) and in rare instances the leaves are reduced to scales (Germishuizen, 2000).

The two species studied belong to different genera, *Sophora* and *Calpurnia*. *Sophora* differs from *Calpurnia* in that their fruits and flowers show some significant differences. In *Sophora* fruit, the seeds within a given pod are at regular intervals (moniliform) and their flowers are white or yellow and rarely blue-violet. *Calpurnia* fruits are winged and have yellow flowers (Germishuizen, 2000).

The Sophoreae is made up of 30 genera and 232 species while the Podalyrieae consists of 26 genera and 125 species (Nkonki *et al*., 2003).



Scheme 1 Phylogeny of the Fabaceae

*Numbers occurring in parenthesis are the number of genera within the tribe.

1.2 Ethnobotanical information of *Sophora* and *Calpurnia*

There has only been one report on the medicinal use of *Calpurnia*, that being the use of *Calpurnia aurea* as an insecticide to kill animal lice (Waka *et al*., 2004). There is no recorded use of *Sophora velutina* in traditional medicine, however other species of *Sophora* have a long list of medicinal uses, with the most popular medicinal use being for its anti-inflammatory, analgesic, antipyretic and anti-cancer properties (Table 1). There are also many other uses listed in Table 1. There has been a substantial amount of literature on the ethnomedicinal uses of *Sophora flavescens* in particular, being used for its antibacterial, antiviral and anti-diarhoeal properties, for skin disorders such as eczema, dermatitis, pyogenic skin infections, carbuncles, scabies, colpitis, leucorrhea, jaundice, hemorrhaging and hepatitis B (Perry and Metzger, 1980; Yoshikawa *et al*., 1985; Tang and Eisenbrand, 1992; Kuroyanagi *et al*., 1999; Kang *et al*., 2000; Ma *et al*., 2002; Ding *et al*., 2006a; Zhang *et al*., 2006; Jeong *et al*., 2008; Jung, 2008). The flowers of *Sophora japonica* has also been used as a blood-staunching agent (Wang *et al*., 2006) and the fruits were reported to have haemostatic properties (Tang *et al*., 2001). In addition, the fruit of *S. japonica* was reported to have anti-fertility effects (Krishna *et al.*, 2012).

Sophora tomentosa is associated with cholera and diarrhoea (Perry and Metzger, 1980) and *Sophora exigua* is used to treat respiratory diseases (Pongboorod, 1950) which could be caused by bacterial infections. *S. flavescens*, *S. grifithi* and *S. tonkinensis* are used as stomachics (Lee *et al*., 2005; Ding *et al*., 2006a,b; Liu *et al*., 2006; Deng *et al*., 2007). *S. flavescens* is also used for gastroenteritis and acute dysentery and *S. subprostrata* is used for peptic ulcers as well as to remove pathogenic heat and to remove toxins (Perry and Metzger, 1980; Sakamoto *et al*., 1992). *Sophora moorcroftiana* is used as a detoxicant (Ma *et al*., 2007). *Sophora alopecuroides* is used

for its sedative and anti-hypothermic properties (Yuan *et al*., 1986). *S. flavescens, S. moorcroftiana* and *S. secundiflora* have been reported to be used for its anthelmintic and parasitic properties (Lee, 1966; Chiang, 1977; Yoshikawa *et al*., 1985; Tang and Eisenbrand, 1992; Huang, 1993; Woo *et al*., 1998; Kuroyanagi *et al*., 1999; Ma *et al*., 2007). *Sophora moorcroftiana* is used as an emetic (Ma *et al*., 2007).

S. flavescens, S. grifithi, S. secundiflora and *S. tonkinensis* are used as diuretics (Lee, 1966; Chiang, 1977; Perry and Metzger, 1980; Yoshikawa *et al*., 1985; Huang, 1993; Liu *et al*., 2006; Deng *et al*., 2007). The use of these plants as diuretics and *S. tomentosa* for hypertension could be related since diuretics are used to control hypertension. *S. viciiffolia* is relatedly used for cystitis and haematuria (Perry and Metzger, 1980; Xiao, 1993) and in addition is used for oedema (Xiao, 1993). *S. tonkinensis* is also used for the treatment of haemorrhoids (Xiao, 1999). *S. flavescens, S. secundiflora* and *S. tomentosa* are all used as antidotes (Chen and Jiang, 1994; Perry and Metzger, 1980). *S. grifith*i has been used as an insecticide and *S. viciifolia* as an antifeedant (Liu *et al*., 2006; Rai, 2006). The seeds of *S. secundiflora* may also have narcotic effects as it is used as a hallucinogenic and emetic during traditional ceremonies (Farnsworth, 1968; Schultes, 1969; 1970).

From the genus *Calpurnia*, only *C. aurea* has been used as an insecticide to kill animal lice in traditional medicine. Table 1 comprehensively cites the plants of *Calpurnia* and *Sophora* and the parts of the plants used in traditional medicine.

Table 1 Species of *Calpurnia* and *Sophora* in traditional medicine

Plant Species	**Part**	**Traditional use**	**Reference(s)**
Calpurnia aurea	leaves	animal Lice	Waka *et al*., 2004
Sophora alopecuroides		sedative, central nervous system depressant, analgesic, hypothermic	Yuan *et al*., 1986
Sophora exigua	roots	antipyretic and respiratory diseases	Pongboord, 1950
Sophora flavescens	roots	antipyretic, analgesic, anti-inflammatory, anthelmintic, stomachic, gastrointestinal, acute dysentery, diarhoeae, antiviral, antibacterial, antidiuretic, eczema, dermatitis, colpitis, hemorrhage; jaundice, leucorrhea, carbuncles, pyogenic skin infections, scabies, enteritis and dysentery, antidote, antifebrile, tumours; hepatitis B; anodyne activities	Lee, 1966; Yoshikawa *et al*., 1985; Tang and Eisenbrand, 1992; Woo *et al*., 1998; Kuroyanagi *et al*., 1999; Kang *et al*., 2000; Chi *et al*., 2001; Ma *et al*., 2002; Lee *et al*., 2005; Ding *et al*., 2006a; Zhang *et al*., 2006; Jeong *et al*., 2008; Jung *et al*., 2008
Sophora grifithii	leafy shoots	stomachic, diuretic, antipyretic, and analgesic properties and insecticide	Liu *et al*., 2006
Sophora japonica	fruits	hemostatic, anti-fertility and anti-cancer activities	Tang *et al*., 2001; Wang *et al*., 2001 & Ma *et al*., 2006
	flowers	blood-staunching agent	Wang *et al*., 2006

Plant Species	Part	Traditional use	Reference(s)
Sophora moorcroftiana	seed decoction	antiphlogistic, detoxicant, emetic, verminosis	Ma *et al*., 2007
Sophora secundiflora	seeds	induce visions (hallucinogenic), ceremonial emetic stimulant	Farnsworth, 1968; Schultes, 1969; 1970
	roots	antipyretic, analgesic, inflammation, sore throat; antidote, antitumor, antiparasitic, diuretic	Chiang, 1977; Chen and Jiang, 1994
Sophora tomentosa		cholera, diarrhoeae, antidote to fish and other marine animal poisoning, hypertension	Perry and Metzger, 1980
Sophora tonkinensis	roots	antipyretic, diuretic, throat inflammation, pain, hemorrhoids, stomachic and anti-tumour agent	Xiao *et al*., 1999; Deng *et al*., 2007
Sophora subprostrata	roots	relieve pain, fever, reduce inflammation, remove toxins, for peptic ulcers and tumours	Son *et al*., 2003; Tingjun and Rongliang, 2004
Sophora viciifolia	roots	fever, cystitis, haematuria, oedema	Xiao, 1993
	roots, bark and seeds	antifeedant	Rai, 2006

1.3 Biological activity of extracts from *Sophora* and *Calpurnea*

In order to find a scientific basis to support the use of plant extracts used in traditional medicine, *in vitro* tests on the extracts of these plants have been carried out. Since most folk medicine made use of water when preparing their prescription, the tests done were mainly on the aqueous or polar extracts such as methanol and ethanol (Table 2). Nonetheless, in a few instances only, hexane extracts were used during the tests.

Of all the plants from the two genera that have been used in alternative medicine, only four plants, *S. flavescens, S. japonica, S. moorcroftiana* and *S. subprostrata* have been subjected to bioassay tests based on their use in traditional medicine. In *Sophora flavescens,* the polar root extracts (methanol and ethanol extracts) have been tested for a variety of biological activity. These tests include antiviral, antioxidant, antiprotozoal, estrogenic, antitoxoplasma and antifeedant assays. The results obtained from these tests are given in Table 2. The tests have confirmed the traditional use of the plant (Table 1). The use of flowers of *Sophora japonica* as a blood-staunching agent in traditional medicine was supported by the test results by Wang *et al*., (2006) (Table 2). There is still a need to validate several of the claims made in Table 1.

Table 2 Pharmacological activities of extracts from *Sophora*

Plant species	Extract	Biological activity	Reference
affinis	aq. EtOH stem and leaf	antitumor	Abbott *et al*., 1966a
angustifolia	aq. EtOH root	antitumor	Abbott *et al*., 1966a
flavescens	aq. root	antiviral	Ma *et al*., 2002
	EtOH root	anti-protozoal; estrogenic	Youn *et al*., 2003; Kim *et al*., 2008b
	MeOH root	cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) inhibitors	Shin *et al*., 2002
	MeOH whole plant	Na^+-glucose cotransporter (SGLT) inhibitory; contact toxicity; anti-*Toxoplasma gondii*; antiprotozoal activity	Youn *et al*., 2003; Liu *et al*., 2007; Sato *et al*., 2007; Choi *et al*., 2008;
	Hexane whole plant	contact toxicity; antifeedant	Liu *et al*., 2007
formosa	aq. EtOH and $CHCl_3$ stem and leaf	antitumor	Abbott *et al*., 1966b
japonica	aq. and EtOH seed and flower	antitumor, tyrosinase inhibition	Abbott *et al*., 1966b; 1966c; 1966d; Wang *et al*., 2006
moorcroftiana	EtOH seed	tumor inhibition rate	Xingming *et al*., 2009
nutalliana	aq. stalk, leaf and fruit	antitumor	Abbott *et al*., 1966e
subprostrata	MeOH whole plant	antiviral	Kim *et al*., 2008a
	aq. and EtOH whole plant	anti-inflammatory, antiulcer and antitumour effects	Son *et al*., 2003
tetraptera	EtOH leaf, flower, stem and fruit	antitumor	Abbott *et al*., 1966a

1.4 A phytochemical review of the lupine alkaloids

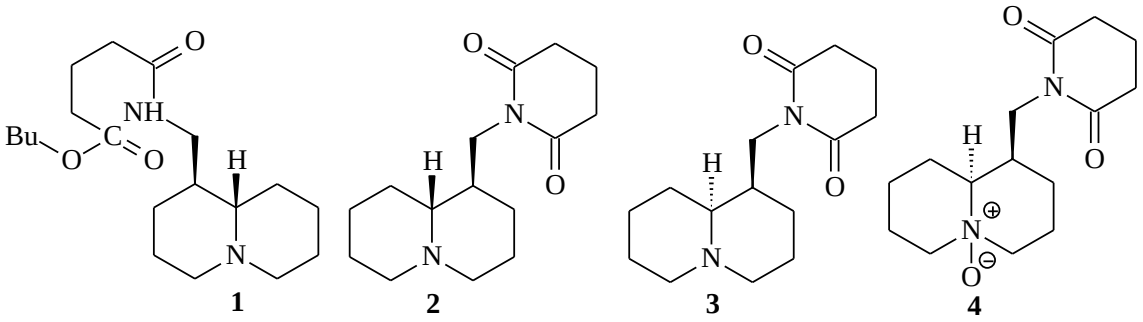
The genera *Sophora* and *Calpurnia* have been studied for its phytochemical constituents as early as 1895. To date there have been over 200 publications on the phytochemistry of species within the two genera with over 470 compounds having been isolated from the genus *Sophora* alone and another 13 being isolated from *Calpurnia* (DNP, 2012; Scifinder, 2012). The most prevalent class of compounds are the flavonoids (400), followed by quinolizidine alkaloids (104) and steroids (50) with some minor compounds of pterocapans, oligostilbenes and benzofurans.

During the course of our study on *Sophora* and *Calpurnia*, both quinolizidine alkaloids and flavonoids were isolated. However, since there are numerous reviews on flavonoids in the literature as well as many series of books, the literature review which follows focuses on the quinolizidine alkaloids only.

1.4.1 Classification of quinolizidine alkaloids

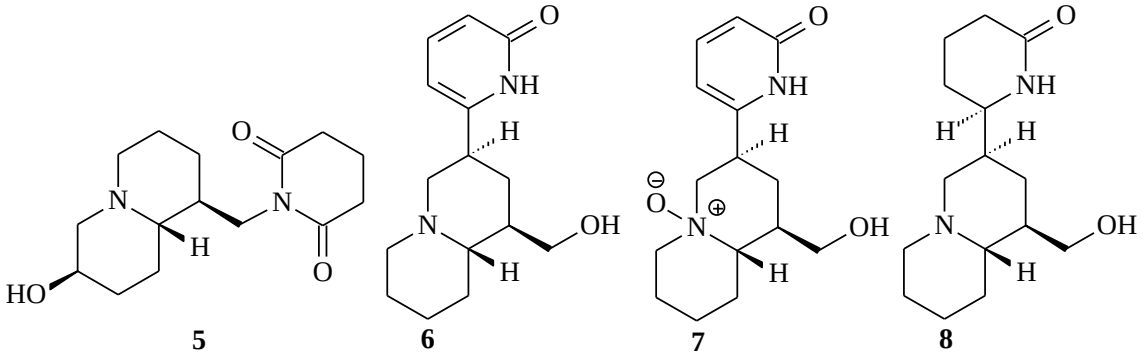
1.4.1.1 Bicyclic quinolizidine alkaloids

These are the simplest form of lupine alkaloids and are typified by lupinine (**9**). Fifteen compounds of this nature have been isolated from these species. Most of these compounds have an additional third six-membered nitrogenous ring.



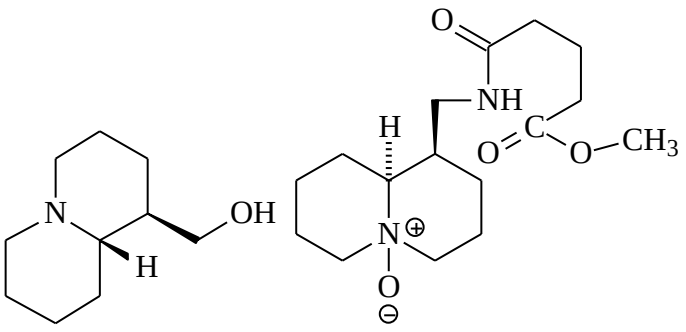
1 **2** **3** **4**

1.	Sophorine
2.	Lamprolobine
3.	Epilamprolobine
4.	N-oxide Epilamprolobine



5 **6** **7** **8**

5.	9β-Hydroxylamprolobine
6.	Mamanine
7.	Mamanine N-oxide
8.	Pohakuline

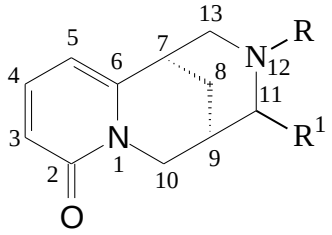


9 **10**

9.	Lupinine
10.	5-(3'-Methoxycarbonylbutyroyl)-aminomethyl-*trans*-quinolizidine N-oxide

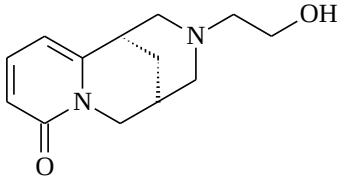
1.4.1.2 Tricyclic quinolizidine alkaloids

Eight tricyclic alkaloids have been isolated from *Sophora* species. These compounds have an additional ring joined to the bicyclic structure in such a manner that a methylene bridge exists between the rings. All these compounds have a characteristic α-pyridone ring A. These alkaloids are typified by cytisine (**11**). These alkaloids can also form dimers for example argentine (**23**).

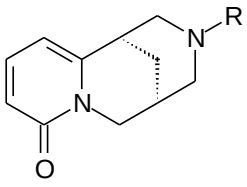


No.	Name	R	R^1
11.	Cytisine	H	H
12.	*N*-Methylcytisine	Me	H

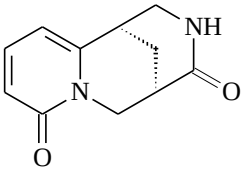
13.	11-Allylcytisine	H	Allyl
14.	*N*-Acetylcytisine	Acetyl	H
15.	*N*-Formylcytisine	Formyl	H



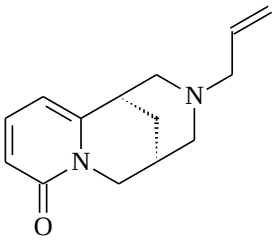
16



17. R=CH_2COOH
18. R=CH_2CONH_2

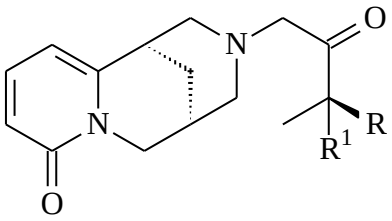


19



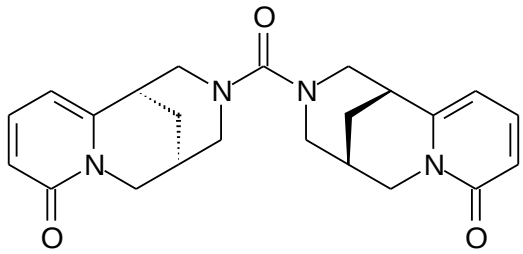
20

16.	*N*-(2-Hydroxyethyl)cytisine
17.	Lehmannine
18.	12-Cytisineacetamide
19.	11-Oxocytisine
20.	Rhombifoline

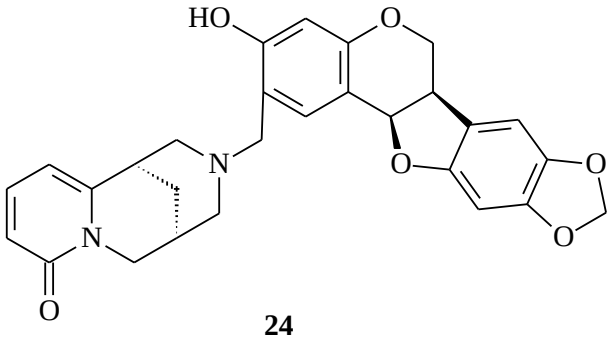


21. R=OH, R^1=H
22. R=H, R^1=OH

21.	Sophorasine A
22.	Sophorasine B

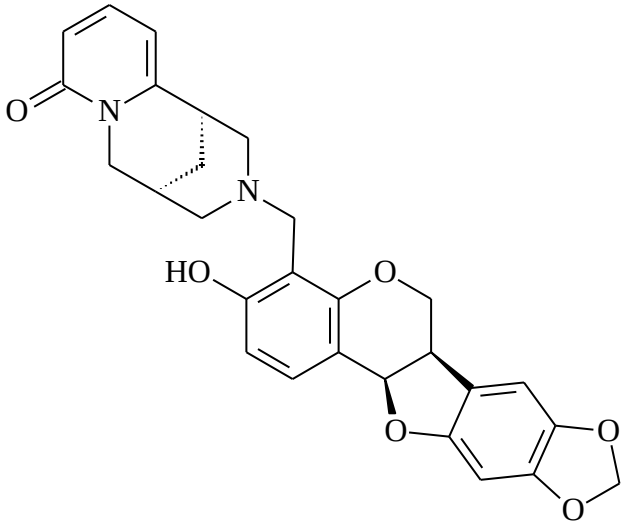


23

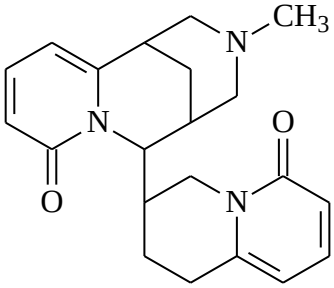


24

23.	Argentine
24.	Tonkinensine A



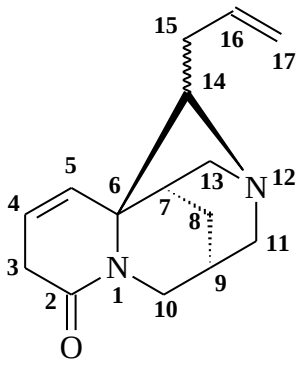
25



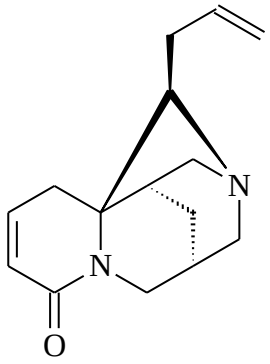
26*

***Compound **26** was published in a thesis without the stereochemistry in all stereogenic centres (Ajaz, 1993)

25.	Tonkinensine B
26.	*N*-Methylsopholupisine



27. 6R:7R:9S:14R
28. 6R:7R:9S:14S



29

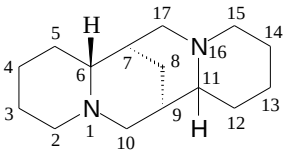
27.	Tsukushinamine A
28.	Tsukushinamine B
29.	Tsukushinamine C

1.4.1.3 Tetracyclic quinolizidine alkaloids

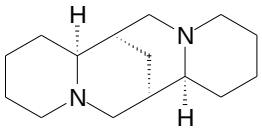
There are two distinct types within the tetracyclic alkaloids. These can be differentiated by whether or not they have a methylene bridge since the manner in which the four rings are fused are different in both types.

The first group is characterized by sparteine (**30**), where a fourth six-membered ring is added onto cytisine (**11**) in a linear fashion to produce a tetracyclic structure. A large number of alkaloids from this group have been isolated from these species. While some of these compounds have the α-pyridone ring, for example thermopsine (**52**), others have a fully saturated ring A as in sparteine (**30**) and some have the amide group retained while losing the double bonds as in lupanine (**35**).

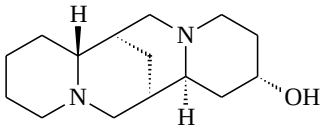
In the second group, the methylene bridge is absent and the four rings are fused in the manner typified by matrine (**63**).



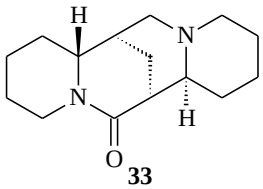
30



31

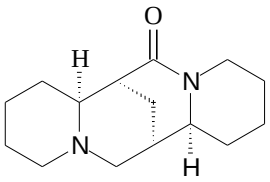


32



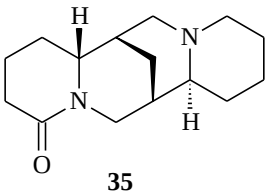
33

30.	Sparteine
31.	β-Isosparteine
32.	13-Hydroxysparteine
33.	10-Oxosparteine

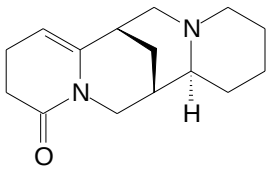


34

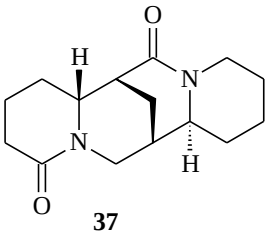
34.	17-oxo-α-isosparteine



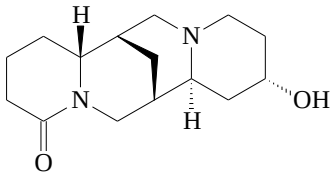
35



36

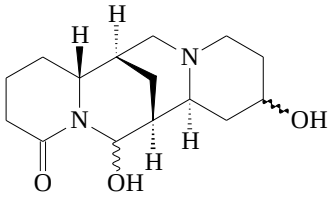


37

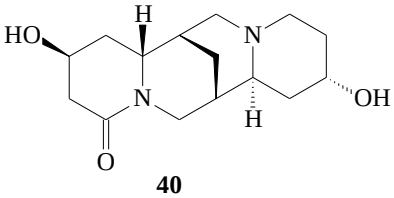


38

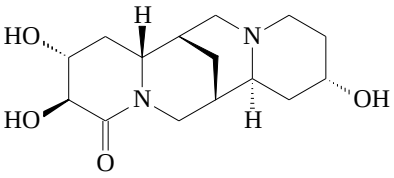
35.	Lupanine
36.	5,6-Dehydrolupanine
37.	17-Oxolupanine
38.	13-Hydroxylupanine



39*



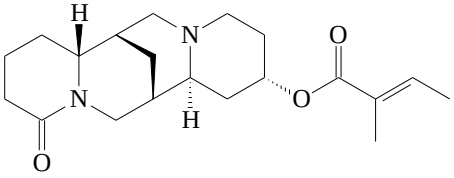
40



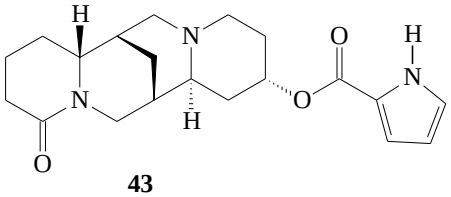
41

***Only the relative stereochemistry is reported in compound **39** (Radema, *et al*., 1979; DNP 2009)

39.	10,13-Dihydroxylupanine
40.	4β,13α-Dihydroxylupanine
41.	3β,4α,13α-Trihydroxylupanine

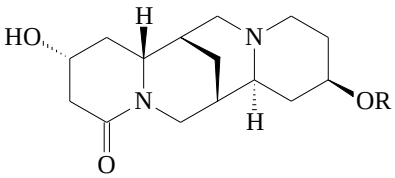


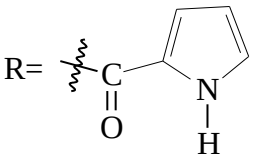
42

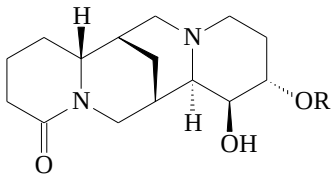


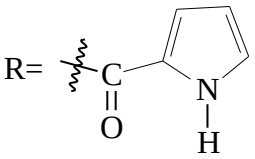
43

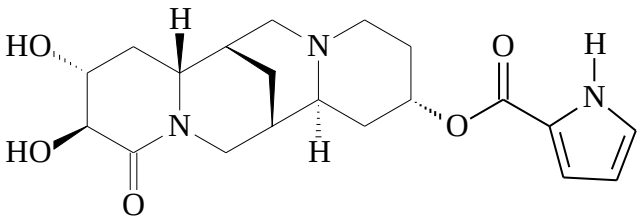
42.	13-Hydroxylupanine tiglate
43.	Calpurnine



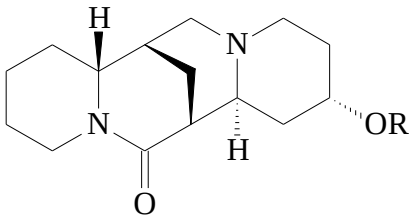
44.	Digittine	
45.	Amino alcohol of digitine	R=H

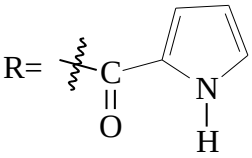


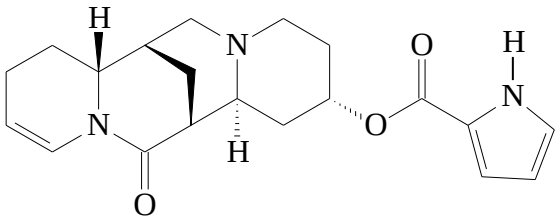
46.	Calpurmenine	R=H
47.	Calpurmenine 13α-pyrrolecarboxylic acid ester	



48.	Calpaurine

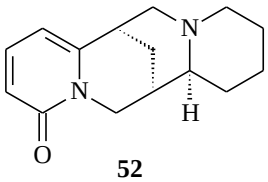


49.	Virgiline	R=H
50.	Virgiline 2-pyrrolecarboxylic acid ester	

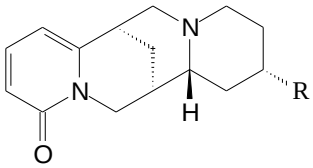


51

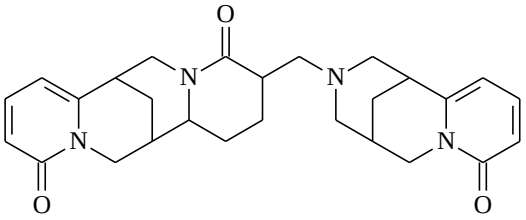
51.	2,3-Dehydro-*O*-(2'-pyrrolylcarbonyl)virgiline



52



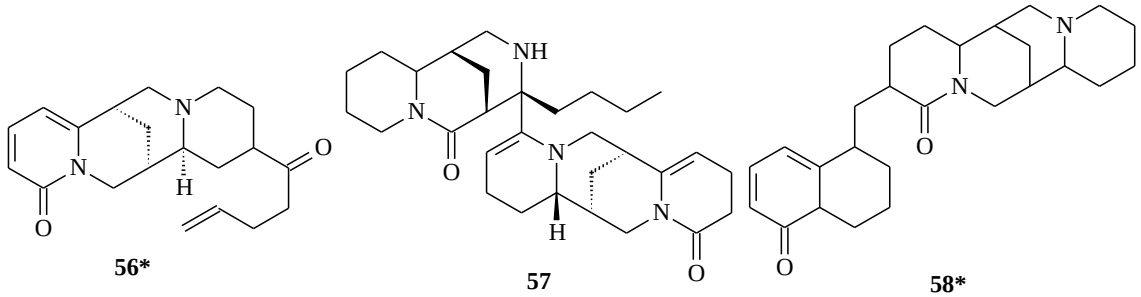
53. R=OH
54. R=H



55*

*****Compound **55** was published in a thesis without the stereochemistry

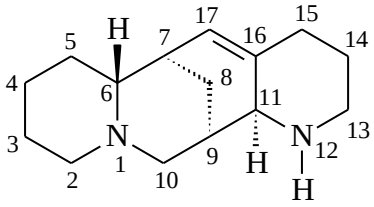
52.	Thermopsine
53.	Baptifoline
54.	Anagyrine
55.	Sophosalimine



56* **57** **58***

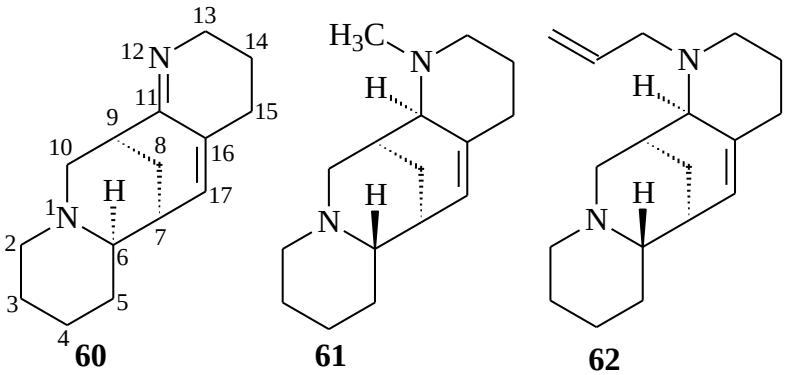
***Compounds **56** and **58** were published in a thesis without the stereochemistry in all stereogenic centres while only the relative configuration was given for **57**.

56.	Sophazrine
57.	Sophohejrine
58.	Sopholupanizidone



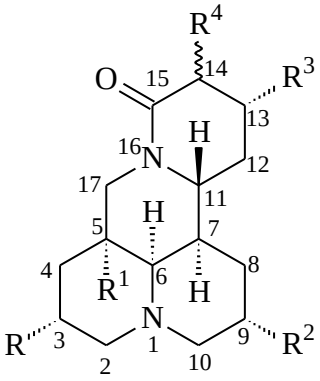
59

59.	Aloperine

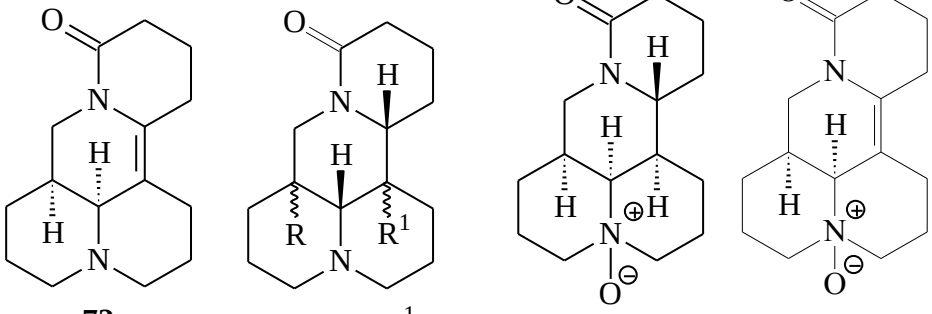


60 **61** **62**

60.	11-Dehydroaloperine
61.	*N*-Methylaloperine
62.	Allylaloperine

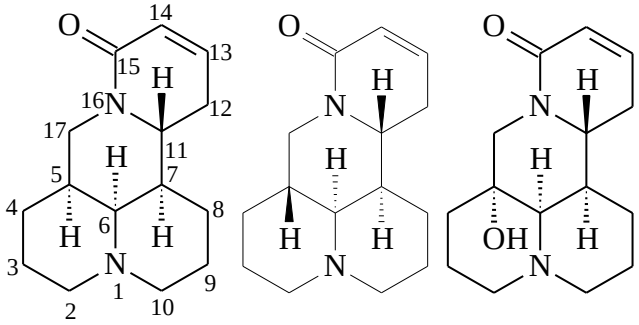


		R	R^1	R^2	R^3	R^4
63.	Matrine	H	H	H	H	H
64.	3α-Hydroxymatrine	OH	H	H	H	H
65.	9α-Hydroxymatrine	H	H	OH	H	H
66.	5α,9α-Dihydroxymatrine	H	OH	OH	H	H
67.	13α-Hydroxymatrine	H	H	H	OH	H
68.	14α-Hydroxymatrine	H	H	H	H	OH
69.	14β-Hydroxymatrine	H	H	H	H	OH
70.	14α-Acetoxymatrine	H	H	H	H	$OCOCH_3$
71.	14β-Acetoxymatrine	H	H	H	H	$OCOCH_3$



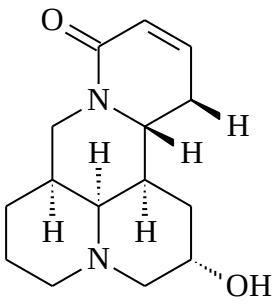
72 **73.** R=β–H, R^1=β–H **74.** R=α–H, R^1=α–H **75.** R=H **76.** R=β-OH **77**

72.	7,11-Dehydromatrine
73.	Isomatrine
74.	Allomatrine
75.	Oxymatrine
76.	14β-Hydroxyoxymatrine
77.	Leontalbinine N-oxide (the N-oxide of 7,11-dehydromatrine)

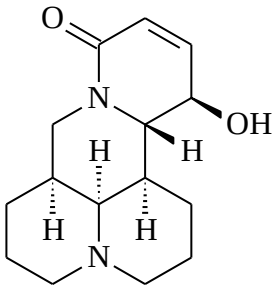


78 **79** **80**

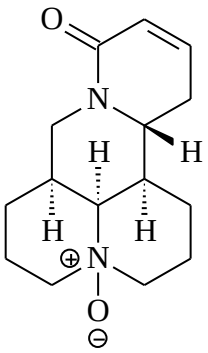
78.	Sophocarpine
79.	5-Episophocarpine
80.	5α-Hydroxysophocarpine



81

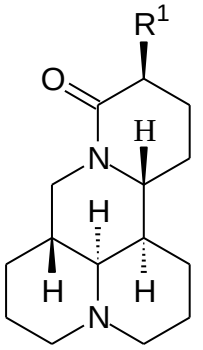


82

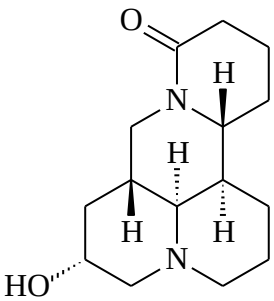


83

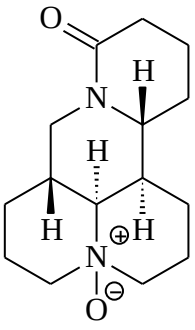
81.	9α-Hydroxysophocarpine
82.	12β-Hydroxysophocarpine
83.	Sophocarpine N-oxide



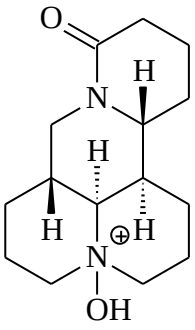
84. R=H
85. R=OH



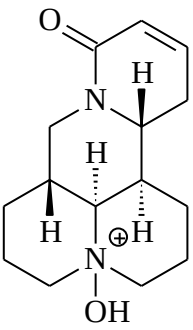
86



87

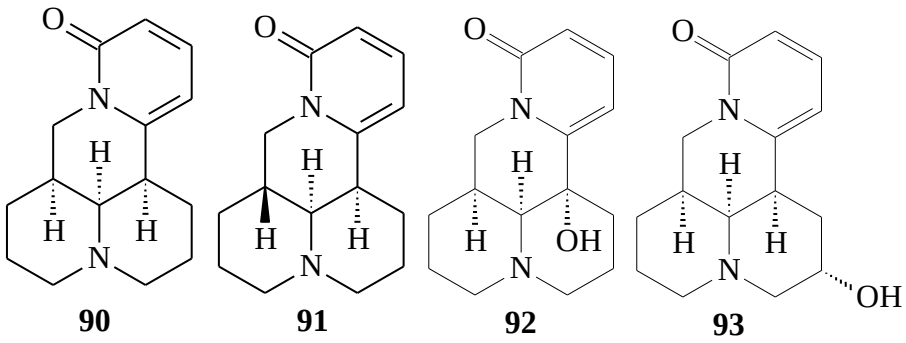


88



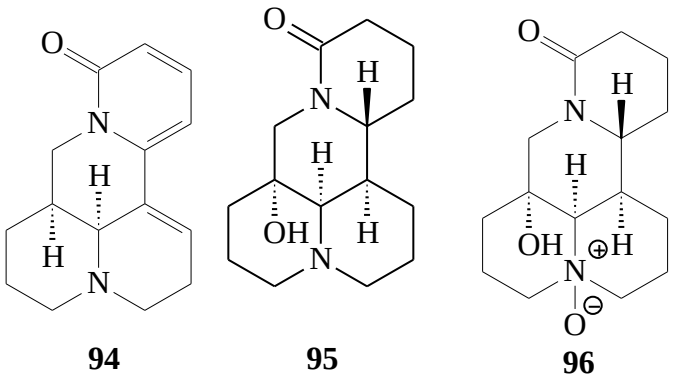
89

84.	Sophoridine
85.	14β-Hydroxysophoridine
86.	3α-Hydroxysophoridine
87.	Sophoridine N-oxide
88.	N-Hydroxysophoridine
89.	N-Hydroxy-13,14-dehydrosophoridine



90 **91** **92** **93**

90.	Sophoramine
91.	Neosophoramine
92.	7α-Hydroxysophoramine
93.	9α-Hydroxysophoramine

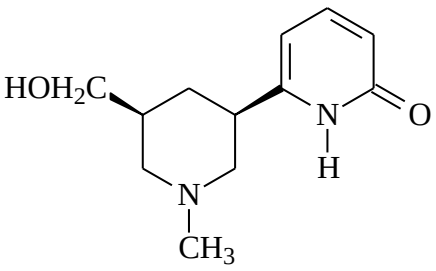


94 **95** **96**

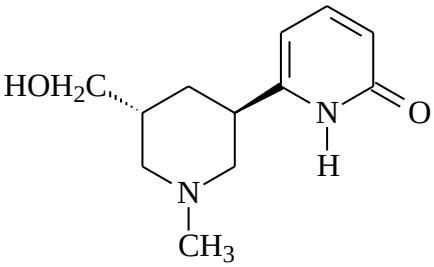
94.	Δ^7-Dehydrosophoramine
95.	Sophoranol
96.	Sophoranol N-oxide

1.4.1.4 Miscellaneous alkaloids

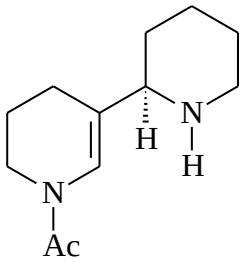
Other alkaloids which have been isolated from these plants could not fall under any of the classes given above and were treated as miscellaneous compounds. These are;



97

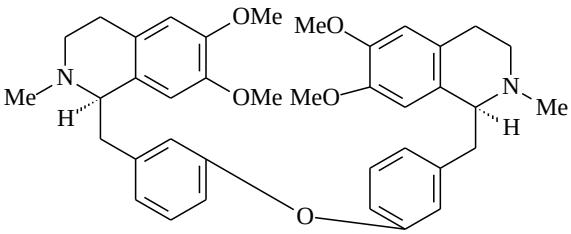


98

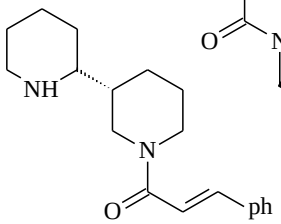


99

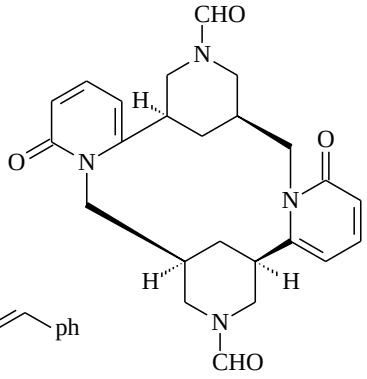
97.	Kuraramine
98.	Isokuraramine
99.	Ammodendrine



100



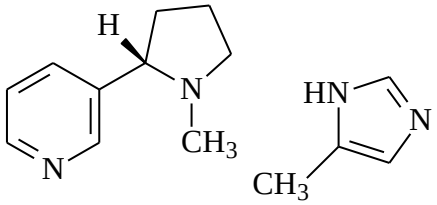
101



102

100.	Dauricine
101.	Adenocarpine
102.	Griffithine

Nicotine (**103**) and 4(5)-methylimidazole (**104**) have also been reported from these plants.



103 **104**

103.	Nicotine
104.	4(5)-Methylimidazole

1.4.2 Quinolizidine alkaloids isolated from *Sophora* and *Calpurnia* species

Plants have been known to accumulate secondary metabolites for different functions and *Sophora* and *Calpurnia* are no exception. Sophora have thirty species with twenty-seven of these having been investigated phytochemically. With the exception of *S. arizonica*, *S. davdii*, *S. fraseri*, *S. koreensis*, *S. leachiana*, *S. moorcroftiana* and *S. stenophylla* which did not contain alkaloids and *S. macrocarpa* which did not contain flavonoids, the rest of the plants from *Sophora* were found to contain both alkaloids and flavonoids together.

Table 3 lists each species of *Sophora* that has been studied phytochemically in alphabetical order with the alkaloids isolated from them. The parts of the plant from where they were found are also included where possible.

The genus *Calpurnia* has not been extensively studied for phytochemical compounds and only two species, *C. aurea* and *C. subdecandra* have been studied phytochemically. These two species have yielded bicyclic and tetracyclic quinolizidine alkaloids (Table 4).

Table 3 Lupine alkaloids from the *Sophora* species

Species	Compound	Reference(s)
Sophora alopecuroides	**1**[ns], **11**[nrs], **16**[ns], **17**[s],**53**[ans], **59**[ns], **60**[l&st], **61**[ns], **62**[ns], **63**[ars], **69**[a], **75**[rs], **78**[anrs], **79**[ns], **82**[a], **83**[rs], **84**[anrs], **86**[ns], **87**[s], **88**[ns], **89**[ns], **90**[anrs], **91**[ns], **92**[a], **101**[a], **103**[s]	Monakhova *et al*., 1973; 1974a; 1974b; Kuchkarov *et al*., 1978; Kamaev *et al*., 1981; Wang *et al*., 1991; Zhang *et al*., 1997; Atta-ur-Rahman *et al*., 2000, Liu *et al*., 2001
Sophora angustifolia	**63**[r]	Plugge, 1895
Sophora chrysophylla	**2**[ste], **3**[ste], **4**[l&ste], **6**[a,b,l&ste], **7**[l], **8**[b], **11**[b,s], **12**[l&ste], **15**[ste], **20**[s], **35**[a,l&ste], **36**[a,l&ste], **37**[l], **53**[s&ste], **54**[s], **63**[b,l&ste], **75**[l&ste], **97**[a,l&ste], **99**[a,l&ste]	Briggs *et al*., 1942; Kadooka *et al*., 1976; Murakoshi *et al*., 1984
Sophora exigua	**18**[r]	Takamatsu *et al*., 1991
Sophora flavescens	**6**[f], **12**[afr], **17**[r], **20**[f], **35**[f], **53**[afr], **54**[afr], **63**[afr], **65**[r], **66**[f], **72**[f], **73**[r], **74**[r], **75**[afr], **77**[s], **78**[afr], **79**[r], **80**[s], **81**[r], **82**[r], **83**[ars], **84**[r], **90**[afr], **93**[fa], **94**[f], **95**[afr], **96**[f], **97**[f], **98**[f], **104**[f]	Bohlmann *et al*., 1958; Okuda *et al*., 1965; Ueno *et al*., 1975, 1978; Morinaga *et al*., 1978; Murakoshi *et al*., 1981a; 1982; Saito *et al*., 1990; Sekine *et al*., 1993; Song *et al*., 1999; Kim *et al*., 2001; Ding *et al*., 2006a
Sophora franchetiana	**11**[ar], **15**[ar], **20**[ar], **27**[a], **28**[a], **29**[a], **53**[ar], **54**[ar], **99**[ar]	Ohmiya *et al*., 1979a; 1979b; 1981
Sophora griffithii	**11**[r], **12**[rs], **21**[l], **22**[l], **23**[s], **26**[ns], **33**[l], **55**[ns], **56**[l], **57**[ns], **58**[ns] **63**[rs], **78**[s], **90**[r], **102**[l]	Primukhamedov *et al*., 1969; 1972; Karakozova *et al*., 1975; Atta-ur-Rahman *et al*., 1991a; 1991b; 1991c; Ajaz, 1993
Sophora japonica	**31**[f]	Keller and Hatfield, 1979
Sophora macrocarpa	**11**[l], **12**[ls], **53**[s], **63**[sl], **64**[l], **65**[l], **75**[l], **95**[l]	Silva, 1968; Negrete *et al*., 1982a; 1982b; 1983
Sophora microphylla	**11**[ba&lw], **12**[ba&l], **54**[l], **63**[ba&lfw], **75**[f], **78**[f]	Briggs *et al*., 1960; 1975; Cui and Zhang, 1986
Sophora pachycarpa	**63**[s], **75**[s], **78**[s], **83**[s], **90**[s]	Zainutdinov *et al*., 1968
Sophora prodani	**11**[s], **30**[sr], **78**[r], **84**[r]	Pislarasu and Badauta-Tocan, 1973; Pislarasu and Dragut,1978

Sophora secundiflora	**8**^{s}, **9**^{l}, **11**^{stl}, **12**^{stl}, **13**^{f}, **14**^{l}, **15**^{stl}, **19**^{l}, **20**^{fst}, **23**^{l}, **30**^{stl}, **31**^{fstr}, **32**^{l}, **35**^{fst}, **36**st, **52**^{s}, **53**^{l}, **54**^{stl}	Izaddoost *et al*., 1976; Keller and Hatfield, 1979; Chavez and Sullivan, 1984; Abdel-Baky and Makboul, 1985; Murakoshi *et al*., 1986; Makboul *et al*., 1987; Abdel-Baky, 1989; Mohamed *et al*., 1993
Sophora subprostrata	**63**^{r}, **76**^{r}	Kojima *et al*., 1970; Cui and Zhang, 1986
Sophora tetraptera	**11**$^{l\&f}$, **12**^{l}, **53**^{l}, **63**^{l}	Reyes *et al*., 1988
Sophora tomentosa	**3**^{alstes}, **4**^{alstes}, **10**^{alstes}, **11**^{alstes}, **12**^{alstes}, **14**^{alstes}, **15**^{alstes}, **53**^{alstes}, **54**^{alstes}, **63**^{alstes}, **75**^{alstes}, **83**^{alstes}, **99**^{alstes}	Ohmiya *et al*., 1974; Komatsu *et al*., 1978; Murakoshi *et al*., 1981b
Sophora tonkinensis	**2**^{l}, **11**^{rns}, **12**^{r}, **15**^{r}, **17**^{rns}, **24**^{r}, **25**^{r}, **35**^{l}, **53**^{l}, **63**^{rnsl}, **65**^{l}, **66**^{l}, **68**^{l}, **69**^{rlns}, **70**^{rl}, **71**^{rl}, **72**^{r}, **74**^{r}, **75**^{rnsl}, **76**^{r}, **78**^{rl}, **80**^{rl}, **83**^{nsrl}, **90**^{ns}, **95**^{nsrl}, **96**^{l}, **100**^{r}	Dou *et al*., 1989; Xiao *et al*., 1996; 1999; Song *et al*., 1999; Ding *et al*., 2005; 2006b; Deng *et al*., 2006; Li *et al*., 2008
Sophora velutina	**2**^{l}, **5**^{l}, **11**^{l}, **12**^{s}	Asres *et al*., 1986a; Koorbanally, 1999
Sophora viciifolia	**11**^{s}, **36**^{s}, **35**^{s}, **63**^{sfl}, **65**^{s}, **67**^{fl}, **69**^{sfl}, **75**^{sfl}, **78**^{s}, **81**^{s}, **82**^{s}, **83**^{sfl}, **84**^{sfl}, **85**^{s}, **90**^{fl}	Zhu *et al*., 1993; Yan *et al*., 1996, Xiao *et al*., 1998

Key: Superscripts, a=aerial parts, b=bark, f=fruits, fl=flowers, l=leaves, r=roots, s=seeds, st=stalks, ste=stem, ns= not specified, where the compounds were isolated.

Table 4 Lupine alkaloids from *Calpurnia* species

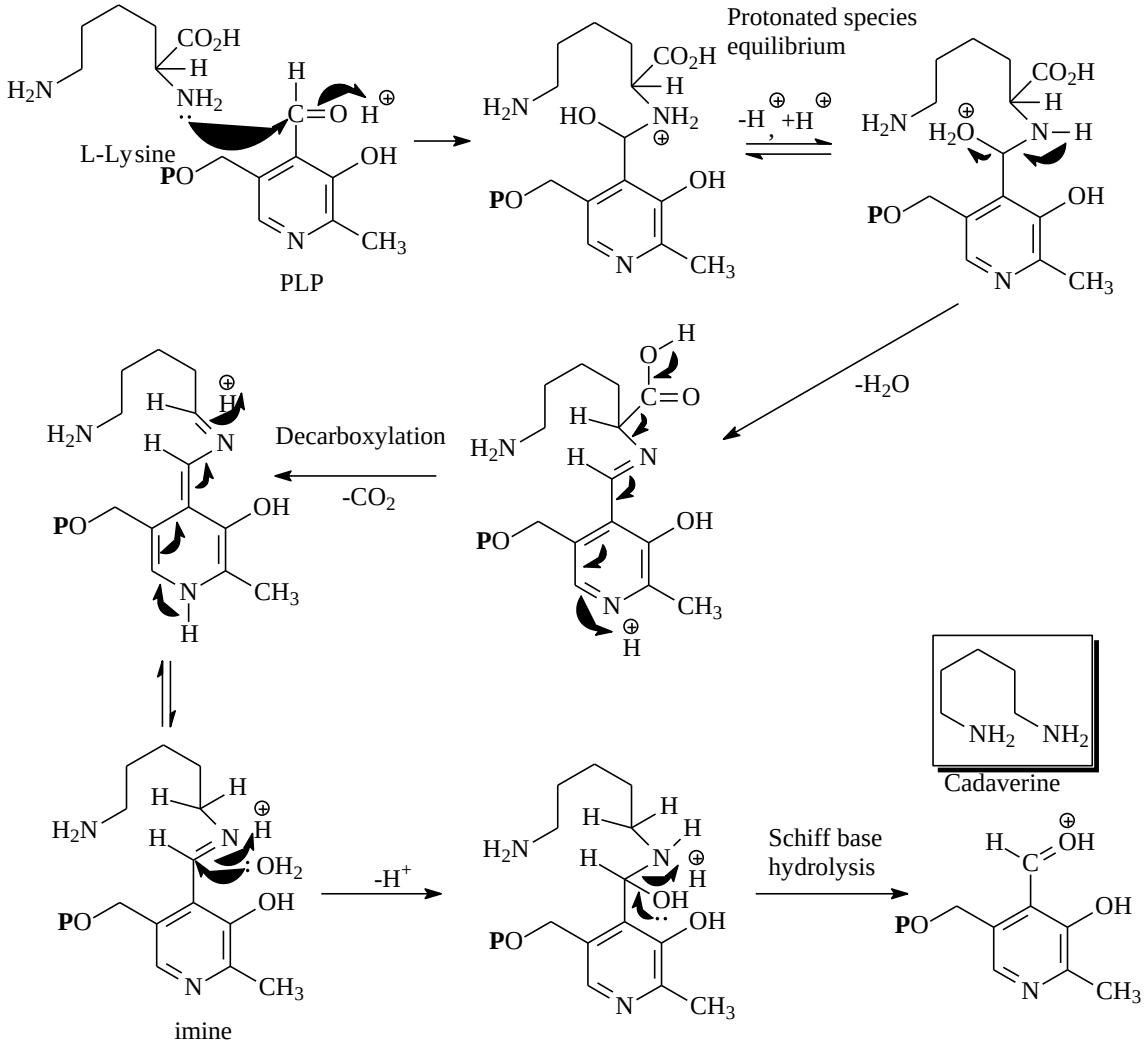
Species	**Compound**	**Reference(s)**
Calpurnia aurea	**8**^{l}, **9**^{l}, **38**^{l}, **39**^{nsl}, **40**^{ns}, **41**^{l}, **43**^{l}, **44**^{ns}, **46**^{lns}, **47**^{lns}, **48**^{l}, **49**^{l}, **50**^{l}, **51**^{l}	Radema *et al*., 1979; Vermin *et al*., 1979; Kubo *et al*., 1984; Asres *et al*., 1986b; 1986c
Calpurnia subdecandra	**43**^{ns}	Goosen, 1963

Key:Superscripts, a=aerial parts, b=bark, f=fruits, fl=flowers, l=leaves, r=roots, s=seeds, st=stalks, ste=stem, ns= not specified, where the compounds were isolated.

1.4.3 Biosynthesis of quinolizidine alkaloids

The phytochemical studies on *Sophora velutina* and *Calpurnia aurea* afforded quinolizidine type alkaloids among other compounds. These alkaloids are made up of slightly varied biosynthetic pathways (van Wyk, 2003) but utilising L-lysine amino acid as the basic building block.

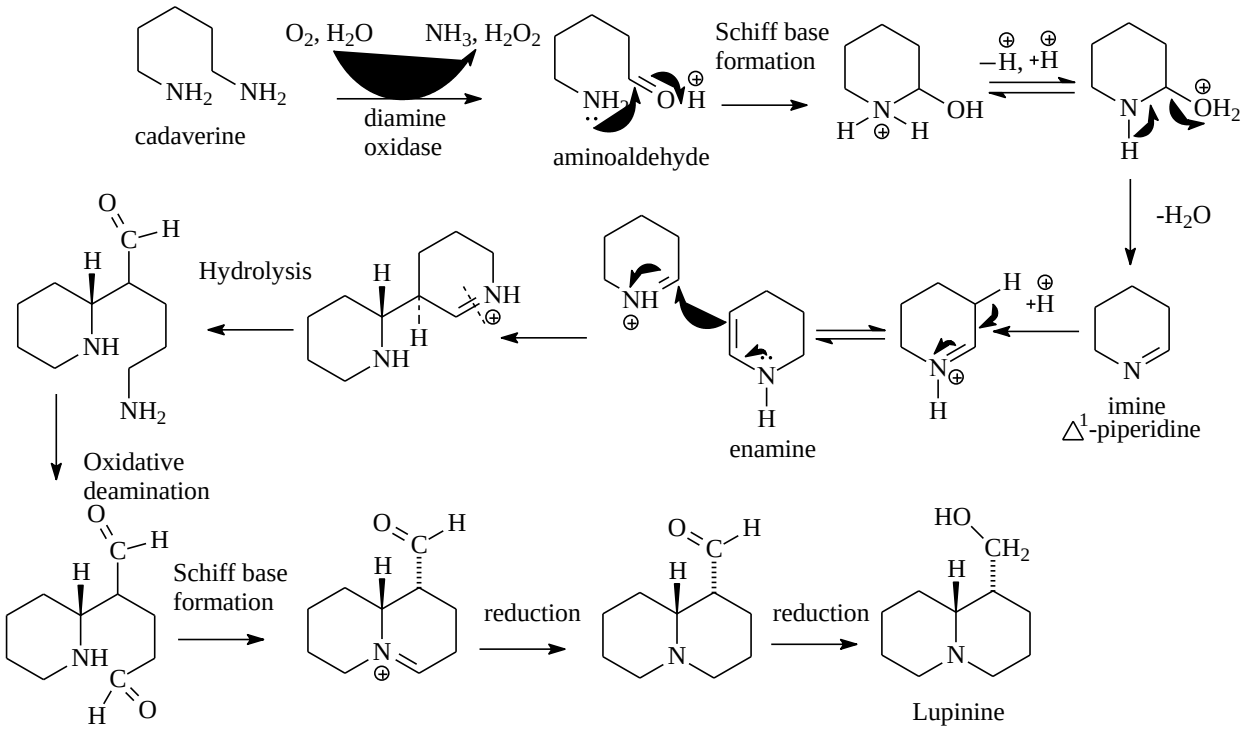
The first step in the biosynthesis of quinolizidine alkaloids is decarboxylation of L-lysine to cadaverine. This step is dependent on the coenzyme pyridoxal phosphate (PLP). The α-amine in L-lysine attacks the carbonyl group in PLP to yield an imine which on undergoing a Schiff hydrolysis gives rise to cadaverine (Scheme 2). This reaction takes place in the enzyme.



Scheme 2 The conversion of L-Lysine to cadaverine (Herbert, 1978; 1980)

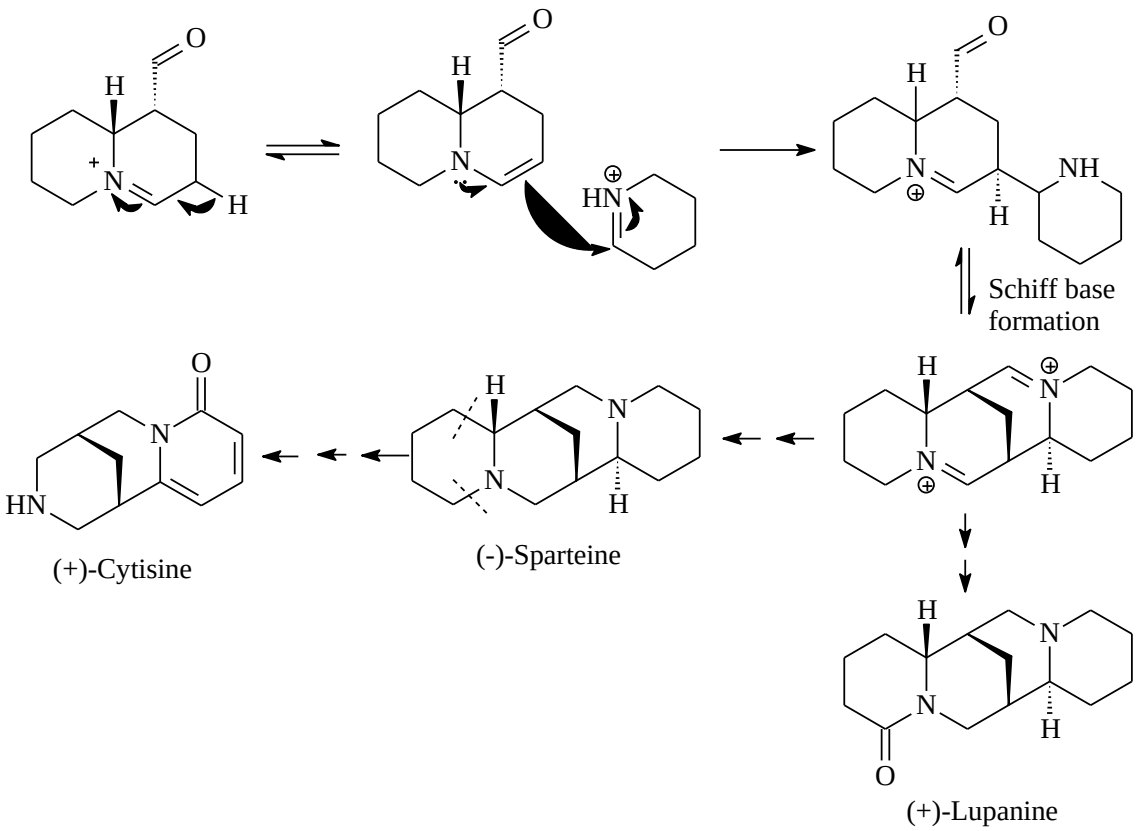
The conversion of cadaverine to an aminoaldehyde is through oxidative deamination. This is done through diamine oxidase and oxygen with the concurrent formation of ammonia and hydrogen peroxide. Aminoaldehyde cyclises to form a cyclic amine (Δ^1-piperideine) which tautomerises via the Δ^1-piperidinium cation to the enamine (Dewick, 2006). The enamine and Δ^1-piperidine form the basic units of bicyclic, tricyclic or tetracyclic alkaloids.

In the biosynthesis of bicyclic alkaloids like (+)-lupinine (Scheme 3), the enamine and Δ^1-piperidinium cation couple with retention of stereochemistry to form an imine which is hydrolysed to an aldehyde followed by oxidative deamination and cyclisation by a Schiff base reaction, then by two reductive steps to yield (+)-lupinine (Dewick, 2006).

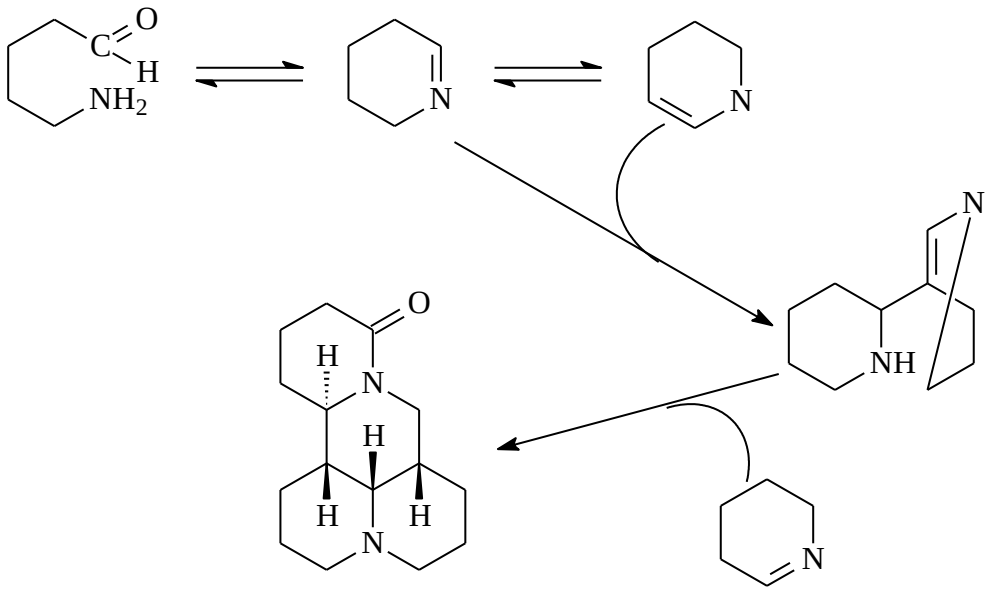


Scheme 3 Biosynthesis of lupinine (Dewick, 2006)

Seyferth *et al*. (1976) and Golebiewski and Spenser (1988) proposed that tetracyclic alkaloids require three Δ^1-piperidine molecules. The arrangement of these three units determines whether a plant synthesises lupanine and sparteine (Scheme 4) or matrine alkaloids (Scheme 5). The cytisine pathway is envisaged to be a result of a loss of the outermost ring from a sparteine molecule followed by oxidation to a pyridone system. Further coupling, hydroxylation and esterification yields hydroxylated and esterified alkaloids (Leeper *et al*., 1981).



Scheme 4 Biosynthesis of lupanine, sparteine and cytisine (Golebiewski and Spenser, 1988)



Scheme 5 Biosynthesis of matrine (Leeper *et al*., 1981)

1.4.4 Biological activity of the quinolizidine alkaloids from *Sophora* and *Calpurnia*

For many years infectious disease have been treated traditionally with plants. Traditionally species of *Sophora* and *Calpurnia* have been used as a remedy for common ailments. It is therefore worth investigating which of the bioactive compounds are responsible for the observed bioactivity. Ethnopharmacological studies to identify antiviral agents from plant material are extensively carried out. It has also been established that compounds with varied structures show similar activities (Ma *et al*., 2002).

It is also interesting to note that **17** lupine alkaloids (Table 5) have been bioassayed and have shown good biological activity. Though not all the studies were based on an ethnobotanical approach in order to find bioactive compounds, some of the compounds have been found to be active against a variety of ailments. Matrine tops the list of these compounds with a variety of pharmacological activities. Tyski *et al*. (1988) found that quinolizidine alkaloids are more active bacteriostatic agents than the normal line antibiotics.

The tests done on these compounds have revealed that ammodendrine (**99**) is the only bycyclic alkaloid that has been reported to have biological activity while the bioactive tricyclic alkaloids are cytisine (**11**), *N*-methylcytisine (**12**) and lehmannine (**17**). In the tetracyclic compounds there are nine matrine types and eight lupanine and sparteine types of alkaloids (Table 5).

Table 5 Compounds isolated from *Sophora* and *Calpurnia* species and their bioactivity

Compound	Biological activity	Reference
Allomatrine (**74**)	Cardiotonic, antiviral	Kimura *et al*., 1989; Ma *et al*., 2002
Aloperine (**59**)	Antifungal, nematicidal	Zhao, 1999; Yu *et al*., 2006
Ammodendrine (**99**)	Teratogenic-crooked calf	Keeler and Panter, 1989
Anagyrine (**54**)	Teratogenic-crooked calf, antiviral, nematicidal-antinematode and anthelmintic activity, toxicity- congenital, malformation in calves	Keeler, 1976; Keeler and Panter, 1989; Ma *et al*., 2002
Cytisine (**11**)	Allelopathy-inhibit seed germination and radicle growth, phe-tRNA binding inhibition and inhibition of *in vitro* wheat germ translation (wheat germ), nematicidal-antinematode and antihelmintic activity, translation *in vitro*, toxicity-teratogenic in chicks and rabbits, antifeeding-mollusc deterance, antiviral.	Wink and Twardouwski, 1992; Ma *et al*., 2002
N-Methylcytisine (**12**)	Hypoglycemic, nematicidal-antinematode and anthelmintic activity, antinematode and anthelmintic activity- motility (spastical), antiviral.	Ma *et al*., 2002; Mohamed *et al*., 1993
Lupanine (**35**)	Allelopathy- inhibit seed germination, antibacterial-growth inhibition, species-specific inhibitory effect, toxicity- *in vitro* inhibition of wheat germ translation, antifeedant, growth inhibitor, antifungal activity –conidia germination inhibition, antifeeding-mollusc deterance, antibacterial- airborne bacteria.	Tyski *et al*., 1988; Wink and Twardowski, 1992; Harborne *et al*., 1998
13α-Hydroxylupanine (**38**)	Antibacterial-growth inhibition, species-specific inhibitory effect, inhibition of *in vitro* wheat germ translation (wheat germ), anti-arryhythmic, hypotensive	Tyski *et al*., 1988; Wink and Twardowski, 1992; Harborne *et al*., 1998

Compound	Biological activity	Reference
13-Tigloyloxylupanine (**42**)	Inhibition of phe-tRNA binding and inhibition of *in vitro* wheat germ translation (wheat germ), allelopathy- inhibit seed germination, antifungal activity –conidia germination inhibition, antibacterial- growth inhibition	Wink and Twardowski, 1992
2,3-Dehydro-O-(2-pyrrolyl-carbonylvirgiline (**51**)	Molluscicidal activity	Kubo *et al*., 1984
Matrine (**63**)	Nematocidal, antipyretic, contractile response of fundis strip, cardiotonic, antinematode and anthelmintic activity-motility (paralytical), glutamate inhibition, antitumor, ehrlich ascites tumor, sarcoma-180 in mouse, antiarrhythmic, anti-inflammatory, antifeedant, anti-cachectic agents, anti-IBD agent, antifibrotic, analgesic, anti-diarrhea, immunosuppressive effects, antifungal, antioxidant activity, anti-hepatitis B virus (HBV), antiviral- liver fibrosis, antiviral	Kojima *et al*., 1970; Yamazaki and Arai, 1985; Cho *et al*., 1986; Kimura *et al*., 1989; Hu *et al*., 1996; 2005; Xin and Ma, 1998; Matsuda *et al*., 1991; Ma *et al*., 2002; Long *et al*., 2004; Xu *et al*., 2004, Cheng *et al*., 2006; Ding *et al*., 2006a,b; Yang *et al*., 2006; Jiang *et al*., 2007; Liu *et al*., 2007; Ma *et al*., 2007; Zhang *et al*., 2008; Ao *et al*., 2009
Isomatrine (**73**)	Antiviral	Ma *et al*., 2002
Oxymatrine (**75**)	Glutamate inhibition, antiviral, antitumor, sarcoma-180 in mouse, anticancer, anti-hepatitis B virus (HBV), anti-inflammatory, antioxidant activity, antifungal, liver injury, antihepatitis C virus, hepatocytes and antihepatic fibrosis	Kojima *et al*., 1970; Ishida and Shinozaki, 1984; Liu *et al*., 1994; 2003; Wang *et al*., 1995; Chen *et al*., 2001; Dong *et al*., 2002; Ma *et al*., 2002; Xiang *et al*., 2002; Ding *et al*., 2006a,b; Yang *et al*., 2006; Ao *et al*., 2009
Sophocarpine (**78**)	Nematocidal, anti-hepatitis B virus (HBV), antitussive in guinea pigs, anticancer, anti-cachectic agents, antiviral	Li *et al*., 1980; Wang *et al*., 1995; Ma *et al*., 2002; Ding *et al*., 2006a,b; Zhang *et al*., 2008
Oxysophocarpine (**83**)	Anticancer, anti-hepatitis B virus (HBV), antiviral	Wang *et al*., 1995; Ma *et al*., 2002; Ding *et al*., 2006a,b
Sophoramine (**90**)	Nematocidal, cardiotonic	Kimura *et al*., 1989
Sophoranol (**95**)	Antiviral	Ma *et al*., 2002

Compound	**Biological activity**	**Reference**
Sophoridine (**84**)	Cardiotonic, antiviral	Kimura *et al*., 1989; Ma *et al*., 2002; Zhang *et al*., 2006
5-Episophocarpine (**79**)	Anti-hepatitis B virus	Ding *et al*., 2006a
Sparteine (**30**)	Allelopathy-inhibit seed germination, allelopathy, antiviral-viral multiplication, inhibition of *in vitro* translation (wheat germ), antimicrobial-growth inhibition, antibacterial-growth inhibition, antimicrobial activity-growth inhibition, antifungal activity –conidia germination inhibition, antifeeding-mollusc deterance, repolarization of neurons exhi, pancreatic β-cell function, antibacterial- airborne bacteria, inhibited charging reaction when ATP & RNA used, species-specific inhibitory effect, oxytoxic agent, adiurectic, hypoglycaemic	Wink, 1987; Tyski *et al*., 1988; Wink and Twardowski, 1992; Harborne *et al*., 1998
10-oxosparteine (**33**)	Insecticidal	Harborne *et al*., 1998
17-oxosparteine (**34**)	phe-tRNA binding inhibition and inhibition of *in vitro* wheat germ translation (wheat germ)	Wink and Twardowski, 1992

Cyclooxygenase (COX), Pathogenic fungi, *Fusarium oxysporum* (FO), *Valsa Pini* (VP), *Cladosporum oxysporum* (CO), *Sphaeropsis sapinea* (SS), *Marssonina brunnee* (MB).

1.5 Aims and objectives of the study

The main aim of the study was to phytochemically investigate two South African species *Sophora velutina* and *Calpurnia aurea* both belonging to the Fabaceae family to investigate whether their use in traditional medicine was justified and whether or not they could provide lead compounds to be used as pharmaceuticals.

The research objectives were;

- To extract and isolate the secondary metabolites present in the fruits and pods, stem and stem bark and leaves of *Sophora velutina* and the leaves, stem and stem bark of *Calpurnia aurea*,
- to identify and characterise the isolated compounds using a range of spectroscopic and other chemical techniques
- to test the compounds in suitable bioassays as determined by the types of compounds that were isolated,
- and to publish the findings of the study in peer reviewed journals.

1.6 References

Abbott, B.J., Hartwell, J.L., Leiter, J., Perdue Jr, R.E., 1966a. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. XXXVIII. Plant extracts. Cancer Research. 26, 1461-1612.

Abbott, B.J., Leiter, J., Hartwell, J.L., Caldwell, M. E., Schepartz, S.A., 1966b. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. XXXIII. Plant extracts. Cancer Research. 26, 587-743.

Abbott, B.J., Leiter, J., Hartwell, J.L., Price, J.R., Perdue Jr., R.E., Schepartz, S.A., 1966c. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. XXXI. Plant extracts. Cancer Research. 26, 207-366.

Abbott, B.J., Hartwell, J.L., Leiter, J., Perdue Jr., R.E., Schepartz, S.A., 1966d. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. XXXVII. Plant extracts. Cancer Research. 26, 1302-1453.

Abbott, B.J., Hartwell, J.L., Iltis, H.H., Kutchen, S.M., Leiter, J., Terdue Jr., R.E., Schepartz, S.A., 1966e. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. XXXVI. Plant extracts. Cancer Research. 26, 1254.

Abdel-Baky, A.M., 1989. Lupine alkaloids from the seeds of *Sophora secundiflora*. Bulletin of the Faculty of Pharmacy, Assiut University. 18, 63-68.

Abdel-Baky, A.M., Makboul, M.A., 1985. Sparteine and 13-hydroxysparteine from the leaves of *Sophora secundiflora* (Orteg.) Lag. Bulletin of Pharmaceutical Sciences. 8, 99-108.

Ajaz, M.S., 1993. Phytochemicial studies of *Sophora griffithii*, *Petchia ceylanica* and *Zizyphus lotus* and synthesis of Asarone analogues. PhD thesis, University of Karachi.

Ao, C., Araki, N., Tawata, S., 2009. Cyclooxygenase inhibitory compounds with antioxidant activities from *Sophora subprostrata*. Asian Journal of Chemistry. 21, 745-754.

Asres, K., Gibbons, W.A., Phillipson, J.D., Mascagni, P., 1986a. The alkaloids of *Sophora velutina*. Journal of Natural Products. 49, 117-121.

Asres K., Phillipson J.D. Mascagni P., 1986b. Two novel minor alkaloids from Ethiopian *Calpurnia aurea* Ssp. a*urea*. Planta Medica. 52, 302-304.

Asres, K., Gibbons, W.A., Phillipson, J.D., Mascagni, P., 1986c. Alkaloids of Ethiopian *Calpurnia aurea* subsp. *aurea*. Phytochemistry. 25, 1443-1447.

Atta-ur-Rahman., Choudhary, M.I., Parvez, K., Ahmed, A., Akhtar, F., Nur-e-Alam, M., Hassan, N.M., 2000. Quinolizidine alkaloids from *Sophora alopecuroides*. Journal of Natural Products. 63, 190-192.

Atta-ur-Rahman., Pervin, A., Feroz, M., Perveen, S., Choudhary, M.I., Hasan, N., 1991a. Isolation and structural elucidation of griffithine by 1D and 2D NMR techniques. Magnetic Resonance in Chemistry. 29, 1077-1083.

Atta-ur-Rahman., Pervin, A., Choudhary, M. I., Hasan, N., Sener, B., 1991b. Sophazrine- a novel quinolizidine alkaloid from *Sophora griffithii*. Journal of Natural Products. 54, 929-935.

Atta-ur-Rahman., Pervin, A., Perveen, S., Nasir, H., Hasan, N., 1991c. Two lupine alkaloids from *Sophora griffithii*. Phytochemistry. 30, 1001-1003.

Bohlmann, F., Rahtz, D., Arndt, C., 1958. Lupine alkaloids. XI. The alkaloids from *Sophora flavescens*. Chemische Berichte. 91, 2189-2193.

Briggs, L.H., Cambie, R.C., Montgomery, R.K., 1975. New Zealand phytochemical survey. 13. Constituents of the wood and bark of *Sophora microphylla* and *Sophora tetraptera*. New Zealand Journal of Science. 18, 555-558.

Briggs, L.H., Russell, W.E., 1942. Sophora alkaloids. III. The alkaloids of seeds of *S. chrysophylla*. Journal of the Chemical Society, 507-509.

Briggs, L.H., Cambie, R.C., Holdgate, R.H., Seelye, R.N., 1960. Sophora alkaloids. VI. The alkaloids of the bark and flowers of *Sophora microphylla* and the isolation of diosmin from the flowers. Journal of the Chemical Society. 1955-1956.

Chavez, P.I., Sullivan, G., 1984. A qualitative and quantitative comparison of the quinolizidine alkaloids of the fascinated and normal stems of *Sophora secundiflora*. Journal of Natural Products. 47, 735-736.

Chen, Y., Li, J., Zeng, M., Lu, L., Qu, D., Mao, Y., Fan, Z., Hua, J., 2001. The inhibitory effect of oxymatrine on hepatitis C virus *in vitro*. Zhonghua Ganzangbing Zazhi. 9, 12–14.

Chen, Y., Jiang, P.C., 1994. A brief survey on studies of *Sophora subprostrata*. Guangxi Medicine. 16, 499–501.

Cheng, H., Xia, B., Zhang, L., Zhou, F., Zhang, Y-X., Ye, M., Hu, Z-G., Li, J., Li, J., Wang, Z-L., Li, C., Guo, Q-S., 2006. Matrine improves 2,4,6-trinitrobenzene sulfonic acid-induced colitis in mice. Pharmacological Research. 53, 202–208.

Chi, Y.S., Jong, H.G., Son, K.H., Chang, H.W., Kang, S.S., Kim, H.P., 2001. Effects of naturally occurring prenylated flavonoids on enzymes metabolizing arachidonic acid: Cyclooxygenases and lipoxygenases. Biochemical Pharmacology. 62, 1185-1191.

Chiang, S., 1977. New medical college, dictionary of Chinese crude drugs, Shanghai scientific technologic publisher, Shanghai, pp 181, 1283.

Cho, C.H., Chuang, C.Y., Chen, C.F., 1986. Study of the antipyretic activity of matrine. A lupin alkaloid isolated from *Sophora subprostrata*. Planta Medica. 52, 343-345.

Choi, K-M., Gang, J., Yun, J., 2008. *Anti-toxoplasma gondii* RH strain activity of herbal extracts used in traditional medicine. International Journal of Antimicrobial Agents. 32, 360–362.

Cui, J., Zhang, G., 1986. Analysis of alkaloid constituents in four *Sophora* species. Zhongyao Tongbao. 11, 102-103.

Deng, Y., Sun, L., Zhang, W., Xu, K., Li, F., Tan, J., Cao, J., Tan, G., 2006. Studies on cytotoxic constituents from *Sophora tonkinensis*. Tianran Chanwu Yanjiu Yu Kaifa. 18, 408-410.

Deng, Y-H., Xu, K-P., Zhou, Y-J., Li, F-S., Zeng, G-Y., Tan, G-S., 2007. A new flavonol from *Sophora tonkinensis*. Journal of Asian Natural Products Research. 9, 45–48.

Dewick, P.M., 2006. Essentials of organic chemistry: for students of pharmacy, medicinal chemistry and biological chemistry. John Wiley and Sons, Inc., Hoboken, New Jersey.

Dictionary of Natural Products, version 21.2; Taylor and Francis Group, 2013; accessed online at http://dnp.chemnetbase.com (Date accessed 21st September 2012).

Ding, P-L., Huang, H., Zhou, P., Chen, D-F., 2006b. Quinolizidine alkaloids with anti-HBV activity from *Sophora tonkinensis*. Planta Medica. 72, 854-856.

Ding, P-L., Liao, Z-X., Huang, H., Zhoub, P., Chena, D-F., 2006a. (+)-12α-Hydroxysophocarpine, a new quinolizidine alkaloid and related anti-HBV alkaloids from *Sophora flavescens*. Bioorganic and Medicinal Chemistry Letters. 16, 1231–1235.

Ding, P-I., Yu, Y-Q., Chen, D-F., 2005. Determination of quinolizidine alkaloids in *Sophora tonkinensis* by HPCE. Phytochemical Analysis. 16, 257-263.

Dong, Y., Xi, H., Yu, Y., Wang, Q., Jiang, K., Li, L., 2002. Effects of oxymatrine on the serum levels of T helper cell 1 and 2 cytokines and the expression of the S gene in hepatitis B virus S gene transgenic mice: a study on the anti-hepatitis B virus mechanism of oxymatrine. Journal of Gastroenterology and Hepatology. 17, 1299–1306.

Dou, J.H., Li, J.S., Yan, W.M., 1989. Studies on the alkaloids of radix *Sophora tonkinensis*. Zhongguo Zhongyao Zazhi. 17, 296-298.

Farnsworth, N.R., 1968. Hallucinogenic plants. Various chemical substances are known to be the active hallucinogenic principles in many plants. Science. 162, 1086-1092.

Germishuizen, G., 2000. Polygonaceae, in: Leistner, O.A. (Ed.), Seed Plants of Southern Africa. Strelitzia. 10, 451-453. National Botanical Institute, Pretoria.

Golebiewski, W.M., Spenser, I.N., 1988. Biosynthesis of the lupine alkaloids. II. Sparteine and lupanine. Canadian Journal of Chemistry. 66, 1734-48.

Goosen, A., 1963. The alkaloids of the Leguminosae. I. The structure of calpurnine from *Calpurnia subdecandra*. Journal of the Chemical Society. 3067.

Harborne, J.B., Baxter, H., Moss, G.P., 1998. Phytochemical dictionary. A handbook of bioactive compounds from plants, 2nd edition. Taylor and Francis. UK.

Herbert, R.B., 1978. In: Barton, D. H. R., Ollis, W. D. (Eds.), Comprehensive organic chemistry, vol. 5. Pergamon, Oxford, pp. 1045.

Herbert, R.B., 1980. In: Coffey, S. (Ed.), Rodd's chemistry of carbon compounds, 2nd edition, vol. IV, Part L. Elsevier, Amsterdam, pp. 291.

Hu, M.J., Zeng, H., Wu, Y.L., Zhang, Y.P., Zhang, S., Qiao, M.M., Fu, H., 2005. Synergistic effects of matrine and 5-fluorouracil on tumor growth of the implanted gastric cancer in nude mice. Chinese Journal of Digestive Diseases. 6, 68–71.

Hu, Z.L., Zhang, J.P., Qian, D.H., Lin, W., Xie, W.F., Zhang, X.R., 1996. Effect of matrine on mouse splenocyte proliferation and release of interleukin-1 and -6 from peritoneal macrophages *in vitro*. Acta Pharmacologica Sinica. 17, 259–261.

Huang, K.C., 1993. The pharmacology of chinese herbs. CRC Press Inc., Boca Raton, FL., pp. 63-66.

ILDIS, 2001. Legumes of the world. International legume database and information service. The University of Reading, UK.

Ishida, M., Shinozaki, H., 1984. Glutamate inhibitory action of matrine at the grayfish neuromascular junction. British Journal of Pharmacology. 82, 523-531.

Izaddoost, M., Harris, B., Gracy, R.W., 1976. Structure and toxicity of alkaloids and amino acids of *Sophora secundiflora*. Journal of Pharmaceutical Sciences. 62, 352-4.

Jeong, G-S., Li, B., Lee, D-S., Byun, E., An, R-B., Pae, H-O., Chung, H-T., Youn, K-H., Kim, Y-C., (2008). Lavandulyl flavanones from *Sophora flavescens* protect mouse hippocampal cells against glutamate-induced neurotoxicity *via* the induction of heme oxygenase-1. Biological Pharmaceutical Bulletin. 31, 1964-1967.

Jiang, H., Hou, C. H., Zhang, S., Xie, H., Zhou, W., Jin, Q., Cheng, X., Qian, R., Zhang, X., 2007. Matrine upregulates the cell cycle protein E2F-1 and triggers apoptosis via the mitochondrial pathway in K562 cells. European Journal of Pharmacology. 559, 98–108.

Jung, H.A., Jeong, D-M., Chung, H.Y., Lim, H.A., Kim, J.Y., Yoon, N.Y., Choi, J.S., 2008. Re-evaluation of the antioxidant prenylated flavonoids from the roots of *Sophora flavescens.* Biological Pharmaceutical Bulletin. 31, 908-915.

Kadooka, M.M., Chang, M.Y., Fukami, H., Scheuer, P.J., Clardy, J., Solheim, B.A., Springer, J.P., 1976. Hawaiian plant studies. XVII. Mamanine and pohakuline, two unprecedented quinolizidine alkaloids from *Sophora chrysophylla*. Tetrahedron. 32, 919-924.

Kamaev, F.G., Kuchkarov, S., Kushmuradov, F.K., Aslanov, K.A., 1981. NMR study of the structure of the alkaloid sophorine from *Sophora alopecuroides*. Khimiya Prirodnykh Soedinenii. 5, 604-608.

Kang, T.H., Jeong, S.J., Ko, W.G., Kim, N.Y., Lee, B.H., Inagaki, M., Miyamoto, T., Higuchi, R., Kim, Y.C., 2000. Cytotoxic lavandulyl flavanones from *Sophora flavescens*. Journal of Natural Products. 63, 680-681.

Karakozova, S.A., Abdusalamov, B.A., Khazanovich, R.L., 1975. Alkaloids from the seeds and pericarp of *Sophora griffithii*. Khimiya Prirodnykh Soedinenii. 5, 664-665.

Keeler, R.F., 1976. Lupin alkaloids from teratogenic and noteratogenic lupins.III, Identification of anagyrine as the probable teratogen by feeding trials. Journal of Toxicology and Environmental Health. 1, 887-898.

Keeler, R.F., Panter, K.E., 1989. Piperidine alkaloid composition and relation to crooked calf disease-inducing potential of *Lupinus formosus*. Teratology. 40, 423-432.

Keller, W.J., Hatfield, M., 1979. 11-Allycytisine and other minor alkaloids from unripe *Sophora secundiflora* fruits. Phytochemistry. 18, 2068-2069.

Kim, H-Y., Shin, H-S., Park, H., Kim, Y-C., Yun Y. G., Park, S., Shin, H-J., Kim, K., 2008a. *In vitro* inhibition of coronavirus replications by the traditionally used medicinal herbal extracts, *Cimicifuga rhizoma*, *Meliae cortex*, *Coptidis rhizoma*, and *Phellodendron cortex*. Journal of Clinical Virology. 41, 122–128.

Kim, I.G., Kang, S.C., Kim, K.C., Choung, E.S., Zee, O.P., 2008b. Screening of estrogenic and antiestrogenic activities from medicinal plants. Environmental Toxicology and Pharmacology. 25, 75–82.

Kim, J.S., Han, S.J., Byun, J.H., Xu, Y.N., Yoo, S.W., Kang, S.S., Son, K.H., Chang, H.W., Kim, H.P., 2001. Minor constituents from the roots of *Sophora flavescens*. Natural Product Sciences. 7, 5-8.

Kimura, M., Kimura, I., Chui, L.H., Okuda, S., 1989. Positive inotropic action and conformation difference of lupine alkaloids in isolated cardiac muscle of guinea pig and bullfrog. Phytotherapy Research. 3, 101-105.

Kojima, R., Fukushima, S., Ueno, A., Saiki, Y., 1970. Antitumor activity of leguminosae plant constituents. I. Antitumor activity of constituents of *Sophora subprostrata*. Chemical and Pharmaceutical Bulletin. 18, 2555-2563.

Komatsu, M., Yokoe, I., Shirataki, Y., 1978. Studies on the constituents of *Sophora* species. XIII. Constituents of the aerial parts of *Sophora tomentosa* L. Chemical and Pharmaceutical Bulletin. 26, 3863-3870.

Koorbanally, N.A., 1999. Extractives from the Amaryllidacea and the Fabaceae, M.Sc dissertation, University of Natal (now University of KwaZulu-Natal), South Africa.

Krishna, P.M., Rao, K.N.V., Sandhya, S., Banji, D., 2012. A review on phytochemical, ethnomedical and pharmacological studies on the genus *Sophora*, Fabaceae. Brazilian Journal of Pharmacognosy. 22, 1145-1154.

Kubo, I., Matsumoto, T., Kozuka, M., Chapya, A., Naoki, H., 1984. Quinolizine alkaloids from the African medicinal plant *Calpurnia aurea*: molluscicidal activity and structural study by 2D-NMR. Agricultural and Biological Chemistry. 48, 2839-2841.

Kuchkarov, S., Kushmuradov, Y.K., Aslanov, K.A., Sadykov, A.S., 1978. Novel quinolizidine alkaloids of *Sophora alopecuroides* L. *Tezisy Dokl. - Sov.-Indiiskii Simp.* Khimiya Prirodnykh Soedineni. 5, 44.

Kuroyanagi, M., Arakawa, T., Hirayama, Y., Hayashi, T., 1999. Antibacterial and antiandrogen flavonoids from *Sophora flavescens*. Journal of Natural Products. 62, 1595-1599.

Lee, S.W., Lee, H.S., Nam, J.Y., Kwon, O.E., Baek, J.H., Chang, J.S., Rho, M.C., Kim, Y.K., 2005. Kurarinone isolated from *Sophora flavescens* Ait inhibited MCP-1-induced chemotaxis. Journal of Ethnopharmacology. 97, 515–519.

Lee, S.J., 1966. Korean Folk Medicine, Publishing Center of Seoul National University, Seoul, pp 84.

Leeper, F.J., Grue-Sorensen, G., Spenser, I.D., 1981. Biosynthesis of the quinolizidine alkaloids. Incorporation of Δ^1-piperideine into matrine. Canadian Journal of Chemistry. 59, 106-115.

Li, X-N., Lu, Z-Q., Qin, S., Yan, H-X., Yang, M., Guan, S-H., Liu, X., Hua, H-M., Wu, L-J., Guo, D-A., 2008. Tonkinesines A and B, two novel alkaloids from *Sophora tonkinensis*. Tetradron Letters. 49, 3797-3800.

Li, Y-Q., Mao, T-F., Yu, S-H., Chao, M-L., Cheng, Y., Wang, C-H., 1980. Pharmacological effects of kugancao (*Sophora alopecuroides*) and sophocarpine. Zhongcaoyao. 11, 555-557.

Liu, B., Li, J., Yuan, Y., 2001. Isolation of alkaloids and structure elucidation of lehmannine from seeds of *Sophora alopecuroides*. Zhongcaoyao. 32, 293-296.

Liu, J., Zhu, M., Shi, R., Yang, M., 2003. *Radix sophorae flavescentis* for chronic hepatitis B: a systematic review of randomized trials. American Journal of Chinese Medicine. 31, 337–354.

Liu, J., Liu, Y., Klaassen, C.D., 1994. The effect of Chinese hepatoprotective medicines on experimental liver injury in mice. Journal of Ethnopharmacology. 42, 183–191.

Liu, J-Y., Hu, J-H., Zhu, Q-G., Li, F-Q., Wang, J., Sun, H-J., 2007. Effect of matrine on the expression of substance P receptor and inflammatory cytokines production in human skin keratinocytes and fibroblasts. International Immunopharmacology. 7, 816–823.

Liu, T-X., Xu, H-H., Luo, W-C., 2006. Opportunities and potentials of botanical extracts and products for management of insect pests in cruciferous vegetables, in: Pelletier, S.W. (Ed.), Advances in phytomedicine, Naturally occurring bioactive compounds, Vol. 3, Elsevier Science, Oxford, pp. 171-191.

Long, Y., Lin, X.T., Zeng, K.L., Zhang, L., 2004. Efficacy of intramuscular matrine in the treatment of chronic hepatitis B. Hepatobiliary and Pancreatic Diseases International. 3, 69-72.

Luo, R., Li, Z., Qian, G., Lu, J., Fu, C., 2009. Determination of Sophoricoside in rat plasma by HPLC and its application to pharmacokinetic studies. Yakugaku Zasshi. 129, 1545-1549.

Ma, S.C., Du, J., But, P.P.H., Deng, X.L., Zhang, Y.W., Ooi, V.E.C., Xu, H.X., Lee, S.H.S., Lee, S.F., 2002. Antiviral Chinese medicinal herbs against respiratory syncytial virus. Journal of Ethnopharmacology. 79, 205-211.

Ma, X. M., Bao, G.S.H., Wan, J.M., Liao, D.J., Yin, S.H.F., Meng, X.Q., Zhou, G.K., Lu, X.M.,. Li, H.Y., 2007. Therapeutic effects of *Sophora moorcroftiana* alkaloids in combination with albendazole in mice experimentally infected with protoscolices of *Echinococcus granulosus.* Brazilian Journal of Medical and Biological Research. 40, 1403-1408.

Makboul, A.M., Abdel-Baky, A.M., Bishay, D.W., 1987. Argentine, lupinine, cytosine and *N*-methylcytisine alkaloids from *Sophora secundiflora* cultivated in Egypt. Bulletin of the Faculty of Pharmacy, Assiut University. 10, 47-54.

Matsuda, K., Yamada, K., Kimura, M., Hamada, M., 1991. Nematicidal activity of matrine and its derivatives against pine wood nematodes. Journal of Agricultural and Food Chemistry. 39, 189-191.

Mohamed, M.H., Kamel, M.S., El-Moghazy, S.A., Murakoshi, I., 1993. The hypoglycemic effect of some lupin alkaloids. Bulletin of the Faculty of Pharmacy (Cairo University). 31, 107-111.

Monakhova, T.E., Proskurnina, N.F., Tolkachev, O.N., Kabanov, V.S., Perel'son, M.E., 1973. Alkaloids of *Sophora alopecuroides*. 3α-Hydroxysophoridine. Khimiya Prirodnykh Soedinenii. 9, 59-64.

Monakhova, T.E., Tolkachev, O.N., Kabanov, V.S., Perel'son, M.E., Proskurnina, N.F., 1974a. Alkaloids of *Sophora alopecuroides*, neosophoramine, a new isomer of sophoramine. Khimiya Prirodnykh Soedinenii. 4, 472-476.

Monakhova, T.E., Tolkachev, O.N., Kabanov, V.S., Proskurina, N.F., 1974b. Alkaloids of *Sophora alopecuroides*. Khimiya Prirodnykh Soedinenii. 10, 259-260.

Morinaga, K., Ueno, A., Fukushima, S., Namikoshi, M., Iitaka, Y., Okuda, S., 1978. Studies on lupin alkaloids. VIII. A new stereoisomer of sophocarpine. Chemical and Pharmaceutical Bulletin, 26, 2483-2488.

Murakoshi, I., Ito, M., Haginiwa, J., Ohmiya, S., Otomasu, H., Hirano, R.T., 1984. Lupine alkaloids from *Sophora chrysophylla*. Phytochemistry. 23, 887-981.

Murakoshi, I., Kidoguchi, E., Haginiwa, J., Ohmiya, S., Higashiyama, K., Otomasu, H., 1982. Isokuraramine and (-)-7,11-dehydromatrine, lupine alkaloids from flowers of *Sophora flavescens*. Phytochemistry. 21, 2379-2384.

Murakoshi, I., Kidoguchi, E., Haginiwa, J., Ohmiya, S., Higashiyama, K., Otomasu, H., 1981a. (+)-Kuraramine, a possible metabolite of (-)-*N*-methylcytisine in flowers of *Sophora flavescens*. Phytochemistry. 20, 1407-1409.

Murakoshi, I., Kidoguchi, E., Nakamuru, M., Haniwa, J., Ohmiya, S., Higashiyama, K., Otomasu, H., 1981b. (-)-Epilamprolobinne and its N-oxide, lupin alkaloids from *Sophora tomentosa*. Phytochemistry. 20, 1725-1730.

Murakoshi, I., Kubo, H., Ikram, M., Israr, M., Shafi, N., Ohmiya, S., Otomasu, H., 1986. (+)-11-Oxocystisine, a lupine alkaloid from the leaves of *Sophora secundiflora*. Phytochemistry. 25, 2000-2002.

Negrete, R., Backhouse, N., Cassels, B.K., 1982a. Leaf alkaloids of *Sophora macrocarpa*. Journal of Natural Products. 45, 652.

Negrete, R.E., Backhouse, N.C., Cassels, B.K., 1982b. Alkaloids from the leaves of the *Sophora macrocarpa*. Boletin de la Sociedad Chilena de Quimica. 27, 263-265.

Negrete, R., Cassels, B.K., Eckhardt, G., 1983 (+)-9α-Hydroxymatrine from *Sophora macrocarpa*. Phytochemistry. 22, 2069-2072.

Nkonki, T., Glen, H.F., Swelankomo, N., Jordaan, M., Germishuizen, G., Moteetee, A., 2003. Fabaceae, in: Germishuizen, G., Meyer, N.L. (Eds.), Plants of southern Africa: An annotated checklist. Strelitzia. 14, 472-559. National Botanical Institute, Pretoria.

Ohmiya, S., Higashiyama, K., Otomasu, H., Haginiwa, J., Murakoshi, I., 1979a. The structure of tsukushinamine, a new type of lupin alkaloid in *Sophora franchetiana*. Chemical and Pharmaceutical Bulletin. 27, 1055-1057.

Ohmiya, S., Higashiyama, K., Otomasu, H., Haginiwa, J., Murakoshi, I., 1979b. The cage-type lupine alkaloids from *Sophora franchetiana* Dunn. Structures of tsukushinamine and its isomers. Koen Yoshishu - Tennen Yuki Kagobutsu Toronkai. 22, 525-531.

Ohmiya, S., Higashiyama, K., Otomasu, H., Haginiwa, J., Murakoshi, I., 1981. Two cage-type lupin alkaloids from *Sophora franchetiana*. Phytochemistry. 20, 1997-2001.

Ohmiya, S., Otomasu, H., Murakoshi, I., Haginiwa, J., 1974. N-Acetylcytisine from *Sophora tomentosa*. Phytochemistry. 13, 1016.

Okuda, S., Murakoshi, I., Kamata, H., Kashida, Y., Haginiwa, J., Tsuda, K., 1965. Lupine alkaloids. I. Minor alkaloids of Japanese *Sophora flavescens*. Chemical and Pharmaceutical Bulletin. 13, 482-487.

Perry, L.M., 1980. Medicinal plants of East and Southeast Asia, MIT press, Cambridge, Massachusetts, London, pp. 226-227.

Perry L.M., Metzger J., 1980. Medicinal plants of east and southeast Asia: Attributed properties and uses, MIT Press, Cambridge, Massachusetts, London pp. 226.

Pislarasu, N., Badauta-Tocan, A., 1973. Alkaloid contents of *Sophora prodani*. Identification and isolation of sparteine. Farmacia. 21, 693-698.

Pislarasu, N., Dragut, E., 1978. Study on the alkaloids content of the *Sophora prodani* Anders species. Farmacia. 26, 121-126.

Plugge, P. C., 1895. Matrine, the alkaloid of *Sophora angustifolia*. Archiv der Pharmazie. 233, 441-443.

Polhill R. M., 1994. Classification of the Leguminosae, in: Bisby, F.A., Buckingham, J., Harborne, J. B. (Eds.), Phytochemical dictionary of leguminosae, XXXV-LVII. Chapman and Hall, New York.

Pongboonrod, S., 1950. Medicinal Plants in Thailand, 1st edition. Kasembunakit Press, Bangkok, pp. 24.

Primukhamedov, I., Aslanov, K.A., Sadykov, A.S., 1969. Content of alkaloids in various *Sophora griffithii* species. Rastitel'nye Resursy. 5, 572-575.

Primukhamedov, I., Aslanov, K.A., Sadykov, A.S., 1972. Alkaloids from the roots of *Sophora griffithii*. Khimiya Prirodnykh Soedinenii. 3, 398-399.

Radema, M.H., van Eijk, J.L., Vermin, W., de Kok, A.J., Romers, C., 1979. Alkaloids of South African samples of *Calpurnia aurea* subsp. *sylvatica*. Phytochemistry. 18, 2063-2064.

Rai, M., Carpinella, M.C., 2006. Naturally Occuring Bioactive Compounds Vol. 3. Advances in Phytomedicine, Elsevier Science and Technology, Netherlands.

Reyes, A., Miranda, N., Martinez, R., 1988. Constituents of *Sophora tetraptera*. *sensu Reiche.* Revista Latinoamericana de Quimica. 19, 32.

Saito, K., Arai, N., Sekine, T., Ohmiya, S., Kubo, H., Otomasu, H., Murakoshi, I., 1990. (-)-5α-Hydroxysophocarpine, a new lupine alkaloid from the seeds of *Sophora flavescens* var. *angustifolia*. Planta Medica, 56, 487-488.

Sakamoto, S., Kuroyanagi, M., Ueno, A., Sekita, S., 1992. Triterpenoid saponins from *Sophora subprostrata.* Phytochemistry. 31, 1339-1342.

Sato, S., Takeo, J., Aoyama, C., Kawahara, H., 2007. Na^+-glucose cotransporter (SGLT) inhibitory flavonoids from the roots of *Sophora flavescens*. Bioorganic and Medicinal Chemistry. 15, 3445–3449.

Schultes, R.E., 1969. Hallucinogens of plant origin. Science. 163, 245-254.

Schultes, R.E., 1970. The botanical and chemical distribution of hallucinogens. Annual Review of Plant Physiology. 21, 571-98.

Scifinder, version 2007.1; Chemical Abstracts Service: Columbus, OH, 2007; accessed online at https://scifinder.cas.org/scifinder (Date accessed 30 August 2012).

Sekine, T., Saito, K., Minami, R., Arai, N., Suzuki, H., Koike, Y., Murakoshi, I., 1993. A new lupine alkaloid, (-)-leontalbinine *N*-oxide, in *Sophora flavescens* var. *angustifolia* seeds and its synthesis by biomimetic transformation from (+)-matrine *N*-oxide. Yakugaku Zasshi. 113, 53-62.

Seyferth, D., Nestle, M.O., Eschbach, C.S., 1976. The biosynthesis of the lupine alkaloids. A reexamination. Journal of the American Chemical Society. 98, 6726-6728.

Shin, H.J., Kim, H.J., Kwak, J.H., Chun, H.O., Kim, J.H., Park, H., Kima, D.H., Lee, Y.S., 2002. A prenylated flavonol, sophoflavescenol: A potent and selective inhibitor of cGMP phosphodiesterase 5. Bioorganic and Medicinal Chemistry Letters. 12, 2313–2316.

Silva, M., Medina, M.V., Sammes, P.G., 1968. Alkaloids of *Sophora macrocarpa*. Phytochemistry. 7, 661-663.

Son, J.K., Park, J.S., Kim, J.A., Kim, Y., Chung, S.R., Lee, S.H., 2003. Prenylated flavonoids from the roots of *Sophora flavescens* with tyrosinase inhibitory activity. Planta Medica. 69, 559-561.

Song, J-Z., Xu, H-X., Tian, S-J., But, P. P-H., 1999. Determination of quinolizidine alkaloids in traditional Chinese herbal drugs by nonaqueous capillary electrophoresis. Journal of Chromatography A, 303–311.

Takamatsu, S., Saito, K., Ohmiya, S., Ruangrungsi, N., Murakoshi, I., 1991. Lupine alkaloids form *Sophora exigua*. Phytochemistry. 30, 3793-3795.

Tang, Y., Lou, F., Wang, J., Zhuang, S., 2001. Four new isoflavone triglycosides from *Sophora japonica*. Journal of Natural Products. 64, 1107-1110.

Tang, W., Eisenbrand, G., 1992. Chinese Drugs of Plant Origin, Springer-Verlag, New York, pp 931.

Tingjun, H., Rongliang, Z., 2004. Promotion of *Sophora subprosrata* polysaccharide on nitric oxide and interleukin-2 production in murine T lymphocytes: implicated Ca^{2+} and protein kinase C. International Immunopharmacology. 4, 109–118.

Tyski, S., Markiewicz, M., Gulewicz, K., Twardowski, T., 1988. The effect of lupine alkaloids and ethanol extracts from seeds of *Lupinus angustifolius* on selected bacterial strains. Journal of Plant Physiology. 133, 240-242.

Ueno, A., Morinaga, K., Fukushima, S., Iitaka, Y., Koiso, Y., Okuda, S., 1975. Lupine alkaloids. VI. Isolation and structure of (+)-isomatrine. Chemical and Pharmaceutical Bulletin. 23, 2560-2566.

Ueno, A., Morinaga, K., Fukushima, S., Okuda, S., 1978. Studies on the lupin alkaloids. VII. Isolation and structure of (-)-Δ^7-dehydrosophoramine. Chemical and Pharmaceutical Bulletin. 26, 1832-1836.

van Wyk, B.E., Schutte, A. L., 1995. Phylogenetic relationships in the tribes Podalyrieae, Liparieae and Crotalarieae, in: Crisp, M., Doyle, J.J. (Eds.), Advances in legume systematics, part 7; phylogeny. Royal Botanic Gardens, Kew, pp. 283-308.

van Wyk, B.-E., 2003. The value of chemosystematics in clarifying relationships in the genistoid tribes of papilionoid legumes. Biochemical Systematics and Ecology. 31, 875–884.

Vermin, W.J., de Kok, A.J., Romers, C., Radema, M.H., van Eijk, J.L., 1979. Calpurmenin and its 13α-(2'-pyrrolecarboxylic acid) ester. Acta Crystallographica, Section B: Structural Crystallography and Crystal Chemistry. B. 35, 1839-1842.

Waka, E.M., Hopkins, R.J., Curtis, C., 2004. Ethnobotanical survey and testing of plants traditionally used against hematophagous insects in Eritrea. Journal of Ethnopharmacology. 95, 95–101.

Wang, X-K., Li, J-S., Omiya, S., Wei, L-X., 1995. The alkaloid constituents in the seeds of *Sophora viciifolia*. Journal of Chinese Pharmaceutical Sciences. 4, 154-156.

Wang, K-H., Lin, R-D., Hsu, F-L., Huang, Y-H., Chang, H-C., Huang, C-Y., Lee, M-H., 2006. Cosmetic applications of selected traditional Chinese herbal medicines. Journal of Ethnopharmacology. 106, 353–359.

Wang, Z., Zhang, S., Fang, S., Zhang, R., Yu, H., 1991. The structure of Δ^{11}-dehydroaloperine. Zhiwu Xuebao. 33, 727-728.

Wink, M., Twardowski, T., 1992. Allelochemical properties of alkaloids. Effects on plants, bacteria and protein biosynthesis, in: Rizvi, S.J.H., Rizvi, V. (Eds.), Allelopathy: Basic and Applied Aspects. Chapman & Hall, London, pp. 129-150.

Wink, M., 1987. Chemical ecology of quinolizidine alkaloids, in: Waller, G.R. (Ed.), Allelochemicals: Role in agriculture and forestry. ACS Symposium Series No. 330, Washington, pp. 524-533.

Woo, E.R., Kwak, J.H., Kim, H.J., Park, H., 1998. A new prenylated flavonol from the roots of *Sophora flavescens*. Journal of Natural Products. 61, 1552–1554.

Xiang, X., Wang, G., Cai, X., Li, Y., 2002. Effect of oxymatrine on murine fulminant hepatitis and hepatocyte apoptosis. Chinese Medical Journal. 115, 593–596.

Xiao, P.G., 1993. A pictorial encyclopaedia of Chinese medical herbs, P, Volume 6, Japanese edition, Chuokoron-Sha, Tokyo, pp. 95.

Xiao, P., Kubo, H., Komiya, H., Higashiyama, K., Yan, Y., Li, J-S., Ohmiya, S., 1999. (-)-14β-Acetoxymatrine and (+)-14α-acetoxymatrine, two new matrine-type lupin alkaloids from the leaves of *Sophora tonkinensis*. Chemical and Pharmaceutical Bulletin. 47, 448-450

Xiao, P., Kubo, H., Komiya, H., Higashiyama, K., Yan, Y-N., Li, J-S., Ohmiya, S., 1998. Lupin alkaloids from seeds of the *Sophora viciifolia*. Phytochemistry. 50, 189-193.

Xiao, P., Li, J., Kubo, H., Saito, K., Murakoshi, I., Ohmiya, S., 1996. 14β-Hydroxymatrine, a new lupine alkaloid from the roots of *Sophora tonkinensis*. Chemical and Pharmaceutical Bulletin. 44, 1951-1953.

Xin, S.M., Ma, Z.Q., 1998. Anti-diarrhea effect of matrine. Chinese Traditional Patent Medicine. 20, 30–32.

Xingming, M., Hongjuan, Y., Ying, D., Yanping, L., Weihua, T., Fangyu, A., Jun, G., 2009. Antitumor effects of ethanolic extracts from *Sophora moorcroftiana* seeds in mice. Iranian Red Crescent Medical Journal. 11, 18-22.

Xu, C.Q., Dong, D.L., Du, Z.M., Chen, Q.W., Gong, D.M., Yang, B.F., 2004. Comparison of the anti-arrhythmic effects of matrine and berbamine with amiodarone and RP58866. Acta Pharmaceutica Sinica. 39, 691–694.

Yamazaki, M., Arai, A., 1985. On the contractile response of fundus strip from rats to matrine, an alkaloidal component of *Sophora flavescens*. Journal of Pharmacobio-Dynamics. 8, 513-517.

Yan, Y., Wang, X., Li, J., Lao, Q., Ji, X., 1996. Studies in the flower of *Sophora viciifolia* Hance. Zhongguo Zhongyao Zazhi. 21, 232-234.

Yang, W., Zeng, M., Fan, Z., Mao, Y., Song, Y., Jia, Y., Lu, L., Chen, C.W., Peng, Y.S., Yang, X-Y., Zhao, B-G., 2006. Antifungal activities of matrine and oxymatrine and their synergistic effects with chlorthalonil. Journal of Forestry Research. 17, 323-325.

Yoshikawa, M., Wang, H.K., Kayakiri, H., Taniyama, T., Kitagawa, I., 1985. Saponin and sapogenol. XL Structure of sophoraflavoside I, a bisdesmoside of soyasapogenol B from Sophorae Radix, the root of *Sophora flavescens* AITON. Chemical and Pharmaceutical Bulletin, 33, 4267-4274.

Youn, H.J., Lakritz, J., Kim, D.Y., Rottinghaus, G.E., Marsh, A.E., 2003. Anti-protozoal efficacy of medicinal herb extracts against *Toxoplasma gondii* and *Neospora caninum.* Veterinary Parasitology. 116, 7–14.

Yuan, H., Yin, Y., He, H., Zhao, Y., 1986. Pharmacological studies on *Sophora alopecuroides*. (II) Neuropharmacological effects of oxymatrine. Yaowu Fenxi Zazhi. 6, 349-352.

Yu, T., Yan, L., Ding, J., Xiao, T., Niu, H., Luo, W., 2006. Bioassay of seven alkaloids from *Sophora alopecuroides* to *Cucumber anthracnose*. Nongyao Kexue Yu Guanli. 27, 23-25.

Zainutdinov, U.N., Aslanov, K.A., Kushmuradov, Y.K. Sadykov, A.S., 1968. *Sophora pachycarpa* seed alkaloids (sophocarpadine). Nauchnye Trudy Tashkentskii Gosudarstvennyi Universitet im. V. I. Lenina. 341, 117-121.

Zhang, L., Li, J., Houghton, P.J., Jackson, S., 1997. Alkaloids in *Sophora alopecuroides* seed and tests for their activities. Zhongguo Zhongyao Zazhi. 22, 740-743.

Zhang, Y., Wang, S., Li, Y., Xiao, Z., Hu, Z., Zhang, J., 2008. Sophocarpine and matrine inhibit the production of TNF-α and IL-6 in murine macrophages and prevent cachexia-related symptoms induced by colon 26 adenocarcinoma in mice. International Immunopharmacology. 8, 1767–1772.

Zhang, Y., Zhu, H., Ye, G., Huang, C., Yang, Y., Chen, R., Yu, Y., Cui, X., 2006. Antiviral effects of sophoridine against coxsackievirus B3 and its pharmacokinetics in rats. Life Sciences. 78, 1998–2005.

Zhao, B-G., 1999. Nematicidal activity of quinolizidine alkaloids and functional group pairs in their molecular structure. Journal of Chemical Ecology. 25, 2205.

Zhu, Z., Zhang, M., Shen, Y., 1993. Antiulcer components of *Sophora viciifolia* alkaloids. Tianran Chanwu Yanjiu Yu Kaifa. 5, 26-29.

Chapter 2. Quinolizidine alkaloids from *Sophora velutina* subsp. *zimbabweensis* (Fabaceae: Sophoreae)

Erick Korir[1], Joyce J. Kiplimo[1], Neil R. Crouch[1,2], Nivan Moodley[3] and Neil. A. Koorbanally[1]*

[1] School of Chemistry and Physics, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa

[2]Ethnobotany Unit, South African National Biodiversity Institute, PO Box 52099, Berea Road, 4007, Durban, South Africa

[3]Biosciences, Centre for Scientific and Industrial Research (CSIR), Pretoria, South Africa

*corresponding author: E-mail address: Koorbanally@ukzn.ac.za Tel.: +2731 260 3189 Fax: +2731 260 3091.

Abstract

Five novel quinolizidine alkaloids, *N*-methylenehydroxycytisine (**A-1**), 7-hydroxylupanine (**A-2**), 6,7-dihydroxylupanine (**A-3**), 7-oxo-thermopsine (**A-4**), and velutinine (**A-5**) have been isolated from the fruits and pods (**A1-A4**) and stem bark (**A-5**) of *Sophora velutina* subsp. *zimbabweensis* along with the known quinolizidine alkaloids, *N*-methylcytisine (**A-6**), cytisine (**A-7**), a cinnamate ester, methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate (**A-8**) and triterpenoids, lup-20(29)-ene-3β-ol (**A-9**) and 12-oleanen-3-one (**A-10**). Compounds **A-10** and **A-6** showed good antibacterial activity against *E. faecalis* with MIC values of 10.9 and 20.8 μg mL^{-1}. The other compounds tested exhibited low to moderate antibacterial activity.

Keywords: *Sophora velutina* subsp. *zimbabweensis*, Fabaceae, *N*-methylenehydroxycytisine, 7-hydroxylupanine, 6,7-dihydroxylupanine, 7-oxo-thermopsine, velutinine.

2.1 Introduction

Quinolizidine alkaloids, found abundant in the Fabaceae, are well-known by the existence of a structural unit in which a nitrogen atom occupies a central position in two fused cyclohexane rings (Hoffmann, 2003). This class of alkaloids are also referred to as lupine alkaloids as they were first discovered in species of the legume *Lupinus* L. In the Fabaceae there are about one hundred and seventy quinolizidine alkaloids that have been isolated and characterised thus far (Aniszewski, 2007), and at times considered chemotaxonomic markers useful in delimiting subfamily groups (e.g. Kite and Pennington, 2003; Pennington *et al.*, 2005). Quinolizidine alkaloids such as matrine and oxymatrine have been reported to possess sedative, depressant, anti-tumour, anti-pyretic, cardiotonic and anti-hepatitis B viral activity (Abbott *et al*., 1966; Kinghorn and Balandrin, 1984).

The genus *Sophora* L., with approximately 50 species, is widespread from southeastern Europe, to temperate Asia, the tropical regions to Australasia and the Pacific. It is poorly represented in Africa (Pennington *et al.*, 2005). Among the African taxa is the large woody shrub *Sophora velutina* Lindl. subsp. *zimbabweensis* Gillett & Brummitt, a highly localised Zimbabwean endemic described relatively recently (Brummitt and Gillett, 1966). This species is quite distinct from other *Sophora* species represented on the African continent, having its strongest affinities with *Sophora velutina* Lindl. var. *albescens* (Rehd.) P.C. Tsoong from the remote mountains of western Szechwan (Brummitt and Gillett, 1966). No ethnomedicinal applications for subsp. *zimbabweensis* in Zimbabwe have been documented (Gelfand *et al*., 1985). Although we have been unable to trace recorded uses in traditional medicine of any of the infraspecific taxa of *S. velutina*, other genus members are so employed, especially in China. The most widely used of these is *Sophora flavescens* Aiton, reputedly for its anti-inflammatory, analgesic, antipyretic, stomachic, anti-cancer, diuretic, anthelmintic,

antibacterial, antiviral and antidiarrhoeal properties. As such, *S. flavescens* preparations are used to treat enteritis, dysentery, respiratory tract infections, leucorrhea, colpitis, jaundice, gastrointestinal hemorrhages, and skin disorders such as scabies, carbuncles, dermatosis and eczema (Chang, 1986; Tang and Eisenbrand, 1992; Huang, 1993; Zhu, 1998; State Pharmacopoeia commission of P.R.C, 2000; Ma, 2002; Liu, 2003).

Phytochemical investigations of several species of *Sophora* have revealed that these plants contain quinolizidine alkaloids. *Sophora velutina* subsp. *zimbabweensis*, the subject of the present study, has previously been phytochemically investigated (Asres *et al*., 1986). These authors isolated three alkaloids from the leaves: a quinolizidine alkaloid (cytisine), and two lupanine-type alkaloids, (+)-lamprolobine and (+)-9β-hydroxylamprolobine. A further well known alkaloid, *N*-methylcytisine was found in the seeds along with two isoflavonoids, pseudobaptigenen and calycosin (Koorbanally, 1997). Such isoflavonoids are also commonly known from the Fabaceae (Dewick, 1994).

The current study was undertaken to isolate natural products (primarily alkaloids) from various *S. velutina* subsp. *zimbabweensis* plant organs and to ascertain their antibacterial activity. This was in view of documented traditional usage profiles of other *Sophora* species, and the known antibacterial activity of various alkaloids (Bruneton, 1995).

2.2 Results and Discussion

Five new alkaloids (Figure 1), *N*-methylenehydroxycytisine (**A-1**), 7-hydroxylupanine (**A-2**), 6,7-dihydroxylupanine (**A-3**) and 7-oxo-thermopsine (**A-4**) from the fruits and pods, and velutinine (**A-5**) from the stem bark have been isolated from *Sophora velutina* subsp. *zimbabweensis.* In addition, the known quinolizidine alkaloids, *N*-methylcytisine (**A-6**) (Wang *et al*., 2000) also from the fruits and pods, cytisine (**A-7**) (Asres *et al*., 1986) from the

leaves, a cinnamate ester, methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate (**A-8**) from the stem bark and triterpenoids, lup-20(29)-ene-3β-ol (**A-9**) (Mahato and Kundu, 1994) from the fruits and pods and 12-oleanen-3-one (**A-10**) (Chiu *et al*., 2008) from the stem bark were isolated. Of these ten compounds, only two, *N*-methylcytisine (**A-6**) and cytisine (**A-7**) have been found previously in *S. velutina* subsp. *zimbabweensis*. The structures of the known compounds were confirmed by 1D and 2D NMR and by comparison with the data published in the literature, except for **A-8** whose structural elucidation was trivial.

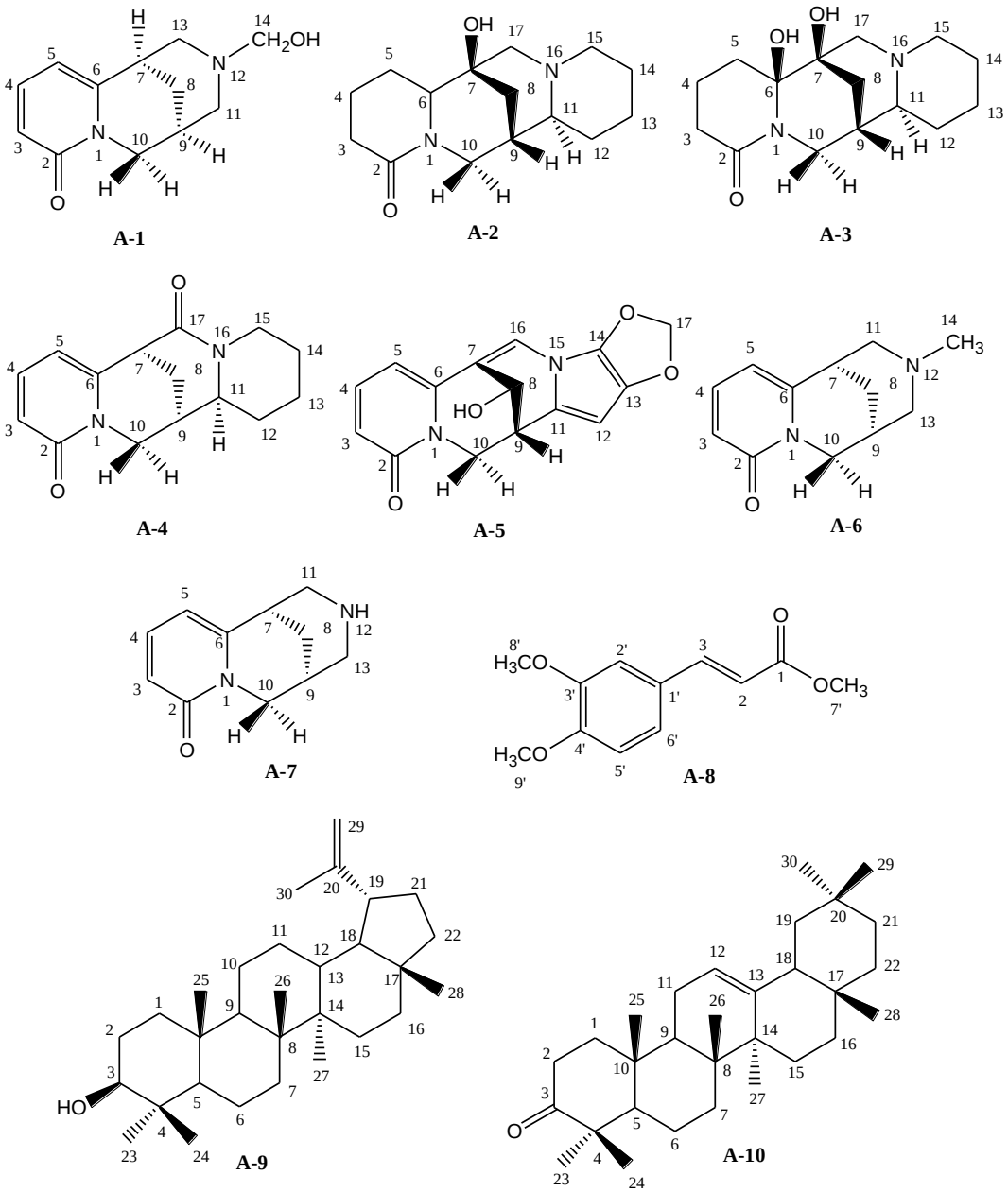


Figure 1 Structures of compounds isolated from *Sophora velutina*

A-1 was obtained as brown crystals. Its IR spectrum exhibited absorption bands at 3424 cm^{-1} (O-H stretch [broad band]), 2931 cm^{-1} (C-H stretch), 1654 cm^{-1} (α,β-unsaturated N-C=O carbonyl stretch), 1560 cm^{-1} (C=C aromatic stretch) and 1431 cm^{-1} (C-N stretch). The ^{1}H and ^{13}C NMR spectra were very similar to that of *N*-methylcytisine with the notable absence of the *N*-methyl singlet resonance at δ_H 2.09 in *N*-methylcytisine and the appearance of a methylene singlet at δ_H 2.69. The *N*-methyl carbon resonance at δ_C 46.15 in *N*-methylcytisine was absent and a methylene carbon resonance at δ_C 79.69 occurred instead. This was indicative that the methyl group in *N*-methylcytisine had been oxidised to a methylenehydroxy group in *N*-methylenehydroxycytisine (**A-1**). The α-pyridone ring was established by resonances at δ_H 7.22 (dd, J = 9.0, 6.8 Hz, H-4), δ_H 6.44 (d, J = 9.0 Hz, H-3) and δ_H 5.63 (d, J = 6.8 Hz, H-5). The characteristic H-10_{ax} and H-10_{eq} resonances could be seen at δ_H 3.82 (dd, J = 15.4, 6.6 Hz) and δ_H 4.00 (d, J = 15.4 Hz). The H-13_{eq} and H-13_{ax} resonances were seen coupled in the COSY spectrum to each other at δ_H 2.60 (J = 10.98 Hz) and δ_H 1.83 (d, J = 10.98 Hz) and the H-11_{eq} resonance overlapped with the H-7 resonance and the *N*-methylenehydroxy proton resonance at δ_H 2.69. The *N*-methylenehydroxy resonance could be distinguished from the other two resonances as it appeared as a sharp intense singlet. Using the COSY spectrum, the other H-11_{ax} resonance was identified at δ_H 2.24 (d, J = 10.81 Hz). The two H-8 resonances were present at δ_H 1.67 (d, J = 12.89 Hz) and δ_H 1.80 (d, J = 12.89 Hz).

The ^{13}C NMR spectrum showed the presence of twelve carbon resonances with five methylene, two methine, three protonated and one non-protonated olefinic resonance and a carbonyl resonance. This suggested the presence of a tricyclic lupane structure. The methylene resonance at δ_C 79.69 is ascribed to the *N*-methylenehydroxy carbon resonance.

The position of the methylenehydroxy substituent at the nitrogen atom is consistent with HMBC correlations between 2H-14 and both C-11 and C-13. The carbonyl resonance at δ_C 163.5 was attributed to the pyridine carbonyl group at position 2 because of HMBC correlations to H-3 and H-4 and the other singlet carbon resonance at δ_C 151.0 to C-6 because of HMBC correlations to H-4, H-5 and H-8_{eq}. The two methine carbon resonances at δ_C 34.7 and δ_C 27.9 were assigned to C-7 and C-9 respectively because of HMBC correlations between C-7 and H-5 and between C-9 and 2H-10. In addition H-10_{eq} showed an HMBC correlation to C-8.

The relative stereochemistry of the molecule was deduced from the NOESY spectrum. The H-7 and H-10 resonances at δ_H 2.71 and δ_H 4.00, respectively, correlate to each other and are both equatorial or alpha. The bridge containing C-8 is in the alpha position together with H-7 and H-9 because correlations between the H-8 resonances and H-7 and H-9 are all seen in the NOESY spectrum. We did not carry out further experiments to determine the absolute stereochemistry since all the sample was used for biological assays.

Unfortunately, the molecular ion could not be detected in the High Resolution Mass Spectrum. We postulate that the *N*-methylenehydroxy group is unstable and cleaves before reaching the detector. This occurs with a concomitant hydrogen transfer to nitrogen resulting in the stable cytisine, whose molecular fragment at *m/z* 190 is seen in the EIMS.

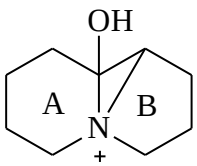
A-2 was isolated as brown oil. Its IR spectrum showed absorption bands at 3427 cm^{-1} (O-H stretch), 2931 cm^{-1} and 2857 cm^{-1} (C-H stretch), and 1672 cm^{-1} (N-C=O carbonyl stretch). The EIMS indicated a molecular ion peak at *m/z* 264 consistent with the molecular formula of $C_{15}H_{24}N_2O_2$. The ^{1}H NMR spectrum showed a three-proton resonance between δ_H 3.65-3.76

(m, 3H) for the two H-10 protons which overlapped with the H-6 proton resonance. The H-17_{eq} resonance appeared as a double doublet at δ_H 2.92 (J = 13.18, 2.57 Hz) and the H-15_{eq} resonance was present as a doublet at δ_H 2.73 (J = 11.35 Hz). Their corresponding axial resonances were present at δ_H 1.96-2.07 (H-15_{ax} overlapping with H-12_{eq} and H-13_{eq}) and δ_H 1.84-1.92 (H-17_{ax} overlapping with 2H-4). The 2H-3/2H-5 proton resonances appeared as an intense triplet at δ_H 2.61 (J = 6.41 Hz). The H-8, H-9, H-11, 2H-14 and the remaining H-12_{ax} and H-13_{ax} resonances all appeared as multiplets between δ_H 0.95-1.60. Their chemical shifts could be determined from the HSQC spectrum by cross correlation with their corresponding carbon resonances.

Although **A-2** had fifteen carbon resonances, only twelve carbon resonances were visible in the ^{13}C NMR spectrum. The teriary oxygenated carbon resonance is assumed to be overlapping with the solvent peak and the C-3 and C-5 resonances overlap as does the C-8 and C-13 resonances, accounting for the three less carbon resonances. There were three methine resonances present at δ_C 66.78, 65.04 and 39.23. Two of these were attributed to the methine carbons attached to nitrogen, C-6 and C-11 and the third assigned to C-9. The C-15 and C-17 methylene carbons attached to N-16 at δ 63.43 and 56.10 respectively were more deshielded than the other methylene resonances, while the C-10 resonance appeared at δ_C 41.55. The other methylene resonances all appeared between δ 17.08 and δ 33.63. The hydroxy group was place at C-7 because COSY coupling between H-6 and H-4 (W coupling) ruled out the possibility that the proton could be situated at C-7. This was further supported by HMBC correlations between H-6 and both C-2 and C-10. The NMR data compare well with both 6-hydroxylupanine **A-11** (Abdel-Halim, 1995) and 6,7-dihydroxylupanine (**A-3**) discussed below (Table 6). It is evident from this table (Table 6) that the C-7 methine carbon is clearly absent and on comparison with **A-3** that the H-6 proton was present.

The relative configuration of the molecule was determined by NOESY correlations between H-9 and H-8. This was consistent with molecular models, which show that the bridge and its substitutents at C-7 and C-9 must have the same orientation. In the absence of a NOESY correlation to H-11, it was assigned as alpha relative to the bridge. Unfortunately we do not have sample to do more experiments to determine the absolute configuration.

A-3 was isolated as a brown oily substance whose IR spectrum showed absorption bands at 3378 cm^{-1} (O-H stretch), 2930 cm^{-1} and 2856 cm^{-1} (C-H stretch), and 1677 cm^{-1} (N-C=O carbonyl stretch). The High Resolution Mass spectrum indicated a mass of 280.1748 consistent with a molecular formula of $C_{15}H_{24}N_2O_3$ (calculated 280.1787). The base peak at *m/z* 154 is a result of the fragment in Figure 2 below, which is consistent with the fragmentation pattern for lupanine but with a hydroxy group at C-6 (Ohmiya *et al*., 1988).



m/z 154

Figure 2 Fragment representing the base peak in the MS of compound **A-3**

The ^1H NMR spectrum showed characteristic resonances for lupanine type alkaloids at δ_H 3.67 (m, H-10eq) and δ_H 3.77 (m, H-10ax), δ_H 2.90 (d, J = 2.0 Hz, H-17eq) and δ_H 2.11 (m, H-17ax), δ_H 2.83 (d, J = 11.6 Hz, H-15eq) and δ_H 2.04 (d, J = 2.38 Hz, H-15ax) as well as for the methylene groups of 2H-3 and 2H-5, which both overlap as a multiplet at δ_H 2.62 and the H-8ax and H-8eq proton resonances which appear at δ_H 0.98 (m) and δ_H 1.45 (m), respectively. The proton resonances at positions 10, 15 and 17 are all deshielded since they

are adjacent to the nitrogen atoms at either position 1 or 16. The ^{1}H NMR resonances compare very well with that of 7-hydroxylupanine (**A-2**) and 6-hydroxylupanine (Abdel-Halim, 1995) with the notable absence of the H-6 and H-7 proton resonances.

The ^{13}C NMR spectrum had twelve visible resonances, with overlapping resonances for C-3 and C-5 at δ_C 32.9. This accounts for thirteen of the fifteen carbon resonances in the molecule, with C-6 and C-7 overlapping with the solvent peak at δ_C 76.7 and δ_C 77.0 accounting for the remaining two resonances. There were two methine carbon resonances in the ^{13}C NMR spectrum at δ_C 39.1 and δ_C 66.5, consistent with that of C-9 and C-11 when compared to 7-hydroxylupanine (**A-2**). Their corresponding proton resonances overlapped at δ_H 2.62 in the ^{1}H NMR spectrum. This proton resonance showed COSY correlations to the two proton resonances of H-10 (equatorial and axial) and H-8 (equatorial and axial) supporting the assignment of H-9. Four carbon resonances at δ_C 27.7, δ_C 39.1, δ_C 66.5 and δ_C 172.7 showed strong HMBC correlations to the proton resonances of H-10ax and H-10eq. One was the carbonyl resonance at C-2 (δ_C 172.7), two were the methine resonances of C-11 (δ_C 66.5) and C-9 (39.1) and the remaining methylene resonance at δ_C 27.7 was assigned to C-8. COSY correlations could also be seen between H-11 and H-12 at δ_H 1.70 and δ_H 1.39 respectively. The resonances of 2H-3 and 2H-5 overlapped at δ_H 2.62 and 2H-4 was present at δ_H 1.88. These assignments were made in comparison with 7-hydroxylupanine and were consistent with HMBC correlations between C-2 and both 2H-3 and 2H-4. The ^{13}C NMR resonances compare well with both 7-hydroxylupanine (**A-2**) and 6-hydroxylupanine (Table 6).

The relative stereochemistry of the molecule was determined using NOESY correlations. In essence, there were NOESY correlations between the axial protons of H-9, H-10, H-8 and H-

17 and between the axial protons of H-11, H-12, H-13 and H-14. NOESY correlations could also be seen between the equatorial protons of H-13, H-14 and H-15 and H-12 and H-10. Due to the small sample size isolated, further experiments to determine the absolute stereochemistry was not possible as all available sample was used for bioassay experiments.

A-4 was isolated as a brown solid. Its IR spectrum showed absorption bands at 2925 (C-H stretch) and 1655 (N-C=O carbonyl stretches). The EIMS indicated a molecular ion peak at *m/z* 258, consistent with the molecular formula of $C_{15}H_{18}N_2O_2$. The ^{1}H NMR spectrum showed resonances typical of quinolizidine alkaloids with an α-pyridone system with the olefinic resonances of H-3, H-4 and H-5 being present at δ_H 6.46, 7.26 and 6.26 respectively with $J_{3,4}$ = 8.97 Hz, $J_{4,5}$ = 6.78 Hz and $J_{3,5}$ = 1.28 Hz, similar to that of cytisine. Also similar to that of cytisine were the resonances of the two H-10 resonances at δ_H 4.22 (d, J = 15.75 Hz, H-10eq) and δ_H 3.91 (dd, J = 15.75, 6.41 Hz, H-10ax). H-9 was identified at δ_H 2.43 by a COSY correlation to H-10ax and the H-7 resonance at δ_H 3.61 showed COSY coupling to the two H-8 protons at δ_H 2.32 and 1.99. The two non-equivalent proton resonances of H-15 appeared at δ_H 4.56 and 2.39, the latter being more shielded due to the shielding effects of the lone pair of electrons on N-16. The H-11 resonance at δ_H 3.32, a doublet with J = 8.79 Hz was seen coupled to the 2H-12 resonance at δ_H 1.60. The H-13ax and the 2H-14 resonances also overlapped at δ_H 1.60.

The ^{13}C NMR spectrum showed the presence of fifteen carbon resonances with two carbonyl resonances at δ_C 166.0 (C-17) and 163.5 (C-2). This was supported by the absence of the methylene carbon, C-17 on comparison with thermopsine. A comparison of the carbon NMR data with both thermopsine (**A-12**) and 17-oxo-sparteine (**A-13**) (Mikhova and Duddeck, 1998) (Table 6) shows that the resonances of C-2 to C-6 match very well with that of **A-12**

due to the similar α-pyridone ring and that C-7 to C-17, the other half of the molecule, match very well with that of **A-13**. This supports the assignment of the extra carbonyl group to C-17. The relative configuration of the molecule was determined by NOESY correlations between H-8 and H-9, H-8 and H-11, and H-9 and H-11.

Table 6 ^{13}C NMR data of 7-hydroxylupanine (**A-2**), 6,7-dihydroxylupanine (**A-3**), and 17-oxo-thermopsine (**A-4**) with 6-hydroxylupanine (**A-11**) (Abdel-Halim, 1995), thermopsine (**A-12**) and 17-oxo-β-isosparteine (**A-13**) (Mikhova and Duddeck, 1998) for comparison

	A-2	**A-3**	**A-11**	**A-4**	**A-12**	**A-13**
2	172.7	172.7	171.6	163.5	163.6	54.6
3	32.9#	32.9#	33.1	118.2	116.4	19.6
4	17.1	17.1	19.4	139.3	138.5	25.3
5	32.9#	32.9#	32.4	106.6	104.4	23.0
6	66.8	76.7*	85.5	144.0	151.6	59.2
7	77.0*	77.0*	37.8	43.2	35.2	43.9
8	27.9##	27.7	15.8	20.6	27.5	20.0
9	39.2	39.1	34.5	32.1	32.8	35.1
10	41.5	41.2	42.8	50.7	44.8	52.9
11	65.0	66.5	63.9	63.5	65.9	61.8
12	33.6	29.0	34.1	33.2	29.7	33.3
13	27.9##	24.1	24.4	25.0	24.3	25.5
14	24.6	24.9	24.6	24.9	25.2	25.6
15	56.1	56.1	55.2	43.9	56.0	42.8
17	63.4	56.6	54.3	166.0	63.3	172.2

* underneath solvent peak; #, ## resonances overlap.

A-5 was isolated as a white crystalline compound. Its IR spectrum showed the presence of a hydroxyl group stretch at 3423 cm^{-1}, a C-H stretch at 2926 cm^{-1}, a C=O stretch at 1618 cm^{-1} and an aromatic C=C stretch at 1508 cm^{-1}. The low stretching frequency of the carbonyl stretch is due to the extended conjugated system present in the molecule. The High Resolution Mass spectrum indicated a mass of 284.0679, consistent with $C_{15}H_{12}N_2O_4$ (calculated 284.0797) and the EIMS showed a fragment at *m/z* 267 which was the loss of a hydroxyl group as well as the molecular ion base peak at M^+ 284.

The proton resonances at δ_H 7.35 (d, J = 8.4 Hz, H-3), δ_H 6.53 (dd, J = 8.4, 2.5 Hz, H-4), and δ_H 6.39 (d, J = 2.5 Hz, H-5) revealed the presence of an α-pyridone ring consistent with that of cytisine (**A-7**). The double doublet of one of the proton resonances on the methylene group at δ_H 3.59 coalesces and appears as a triplet with $J_{9,10ax}$ of 11.0 Hz being equal to $J_{10ax,10eq}$. The resonance was assigned to the axial position because it experiences the full effect of the nitrogen lone pair on the alpha face of the molecule, shielding this proton resonance more than its equatorial counterpart (Wiewiorowski *et al*., 1967) present at δ_H 4.20 (dd, J = 11.0 Hz, 5.0 Hz) as indicated in the HSQC spectrum. For the more stable chair conformation to exist with rings B and C, the nitrogen lone pair must face away from the bridge. The molecule therefore has a relative configuration of the bridge being *beta* and the lone pairs on the two nitrogen atoms being *alpha*. The axial H-10 proton is also *alpha* and the equatorial H-10 proton *beta*. As with the other samples, the absolute configuration was not determined due to the sample being used for biological assays.

Both the H-10 resonances showed a COSY correlation with the multiplet at δ_H 3.47 (H-9) which is more downfield than those observed in cytisine and *N*-methylcytisine where H-9 resonated between δ_H 2.32 and δ_H 2.40. The H-9 proton also showed a COSY correlation with the oxygenated methine resonance at δ_H 5.45 (d, J = 7.0 Hz) which was attributed to H-8. This was supported by HMBC correlations between H-8 and both C-6 and C-10.

There were two other more deshielded singlet resonances at δ_H 6.41 and δ_H 6.70. Both these resonances showed HMBC correlations to the resonances at δ_C 154.2 (C-11) and δ_C 148.1 (C-14), with δ_H 6.70 showing an additional correlation to δ_C 141.7 (C-13) and δ_H 6.41 showing an additional correlation to δ_C 112.6 (C-7). This prompted δ_H 6.70 to be assigned to H-12 and

the resonance at δ_H 6.41 to H-16. HMBC correlations between H-9 and both C-7 and C-11 supported these assignments.

The C-2 and C-6 resonances, both at approximately the same chemical shift were distinguished by HMBC correlations to H-3 and H-8 respectively. The carbon signal at δ_C 101.1 and its corresponding proton resonances at δ_H 5.90 and δ_H 5.87, both doublets (1.5 Hz) was consistent with that of a methylenedioxy group which was placed at C-13 and C-14 in the molecule since HMBC correlations was seen between both these non-equivalent resonances to both C-13 and C-14. The more deshielded carbon signal was assigned to C-14 due to the inductive electron withdrawing effect of both the oxygen and the nitrogen. This is the first report of compound **7** and we have given it the trivial name velutinine.

The results of the Minimum Inhibitory Concentration (MIC) determinations of the samples against against *Enterococcus faecalis* and *Pseudomonas aeruginosa* are given in

Table 7. *P. aeruginosa* showed resistance against eight of the ten samples tested with only **A-8** and **A-10** being slightly active at 200 and 175 μg mL^{-1}, respectively. **A-10** (the steroidal ketone, 12-oleanen-3-one) and **A-6** (the quinolizidine alkaloid, *N*-methylcytisine) showed good antibacterial activity against *E. faecalis* with MIC values of 10.9 and 20.8 μg mL^{-1} respectively. Two other samples, an aromatic ester (**A-8**) and the lupane alkaloid, 17-oxo-thermopsine (**A-4**) showed moderate antibacterial activity against *E. faecalis* at concentrations of 100 and 125 μg mL^{-1}, respectively.

Table 7 MIC values of the isolates from *S. velutina* subsp. *zimbabweensis* against *E. faecalis* and *P. aeruginosa*

Compound	Average MIC (μg mL^{-1})	
	Enterococcus faecalis	*Pseudomonas aeruginosa*
A-1	>250.00	>250.00
A-2	>250.00	>250.00
A-3	208.33	>250.00
A-4	125.00	>250.00
A-5	>250.00	>250.00
A-6	20.83	>250.00
A-7	>250.00	>250.00
A-8	100.00	200.00
A-9	>250.00	>250.00
A-10	10.90	175.00
Gentamicin	0.39	0.78

2.3 Conclusions

While most of the compounds isolated were inactive against both *E. faecalis* and *P. aeruginosa*, two compounds, *N*-methylcytisine (**6**) and 12-oleanene-3-one (**10**) showed good activity against *E. faecalis*. This activity could be due to the *N*-methyl group in the quinolizidine alkaloid or the 3-keto group in the steroidal ketone. These compounds could make interesting subjects for structure activity relationship studies with *E. faecalis*.

2.4 Experimental section

General experimental procedures

IR spectra were recorded on a Perkin-Elmer Universal ATR Spectrometer and UV spectra on a Varian Cary UV-VIS Spectrophotometer. Specific rotations were measured at room temperature in methanol on a Perkin-Elmer^{TM}, Model 341 Polarimeter with a 10 mm flow tube. The melting points were recorded on an Ernst Leitz Wetzer micro-hot stage melting point apparatus. The ^{1}H, ^{13}C and all 2D NMR spectra were recorded using a Bruker Avance^{III} 400 MHz spectrometer. Spectra were recorded at room temperature using either deuterated

methanol (CD_3OD) or deuterated chloroform ($CDCl_3$) as solvent. For GC-MS analyses, the samples were analysed on an Agilent GC–MSD apparatus equipped with DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 μm film thickness) fused-silica capillary column. Helium (at 2 ml/min) was used as a carrier gas. The MS was operated in the EI mode at 70 eV. High Resolution Mass Spectrometry was carried out by UPLC-DAD-MS with a Waters SYNAPT HDMS system (4KDA) consisting of a sample manager, ultra-pressure binary pump, integrated column oven and DAD detector connected in series to a SYNAPT G1 QTOF mass spectrometer and equipped with an Acquity HSS T3 Waters column (1.8 μm, 150 x 2.1 mm). The system was controlled through MassLynx v 4.1 SCN639. The gradient programme used was as follows: 5% (v/v) aqueous HPLC-gradient acetonitrile (A) in 0.1% (v/v) formic acid increasing to 90% acetonitrile over 15 min.

The separation, isolation and purification of compounds were carried out by gravity column chromatography using Merck silica gel 60 (0.040-0.063 mm) and monitored by thin layer chromatography (TLC; Merck 20 × 20 cm silica gel 60 F_{254} aluminum sheets). The extracts were crudely separated on a 4 cm diameter column using appropriate solvent systems which gave the best separation on TLC. Fraction sizes of 100 mL each were collected and twenty fractions (a total of 2 L) were collected for each stage.

Plant collection

Fruits (including the pods), stems (including the bark) and leaves of *Sophora velutina* Lindl. subsp. *zimbabweensis* Gillet & Brummit were obtained from a plant cultivated in Pretoria, South Africa. A voucher specimen (*Crouch 780*) was deposited at the KwaZulu-Natal Herbarium (NH) in Durban, South Africa.

Extraction and Isolation

The air dried plant parts (fruits, pods and leaves) were grounded in a domestic blender or milled (stem and bark) and then extracted separately using a soxhlet apparatus with hexane, dichloromethane, ethyl acetate and methanol successively for 24 hours each. Extraction of 750 g of the fruits and pods yielded 47.7 g, 20.0 g, 28.4 g and 68.3 g of hexane, dichloromethane, ethyl acetate and methanol extracts respectively. Extraction of 640 g of the leaves and 703 g of the stem and bark yielded 48.2 g, 9.3 g, 26.2 g and 83.9 g (leaves) and 7.0 g, 2.4 g, 7.9 g, and 3.0 g (stem and bark) for the four solvents mentioned above, respectively.

Isolation of compounds from the fruits and pods

The hexane extract was eluted with 2 litres each of a hexane:dichloromethane step gradient 100:0, 90:10, 80:20, 70:30, 60:40, 0:100 and then 1% and 2% methanol in dichloromethane. Fractions 91-95 were combined and further purified on a 1 cm diameter column with 35% dichloromethane in hexane, collecting 2 mL fractions each. Lup-20(29)-ene-3β-ol (**A-9)** (25.0 mg) eluted in fractions 24-45.

The dichloromethane extract was eluted with a hexane:dichloromethane step gradient as for the hexane extract, followed by 1%, 2%, 3% and 5% methanol in dichloromethane. Fractions 90-92 was purified further with 2% methanol in dichloromethane, where *N*-methylcytisine (**A-6)** (21.5 mg) eluted in fractions 4-42. Fractions 181-189 was purified with 3% methanol in dichloromethane where fractions 5-24 contained thermopsine (**A-4**) (23.3 mg).

The methanol and ethyl acetate extracts were combined (as a TLC analysis showed that they contained similar components) and dissolved in a 1:1 mixture of methanol:water (500 ml in total). This solution was then acidified with 4 M HCl to pH 4 and extracted with 3 x 250 mL

portions of chloroform. The chloroform extracts were combined and evaporated under reduced pressure to produce 67.1 g of extract A. The aqueous phase was then basified to pH 9 using 4 M NH_4OH and extracted with 3 x 250 mL portions of chloroform. The combined chloroform extracts yielded 5.6 g of extract B. Extract A did not yield any compounds of interest on separation.

Extract B was separated on a 2 cm column with a methanol:dichloromethane step gradient of 100% dichloromethane (500 ml) and then 1L each of 2%, 4%, 6%, 8% and 10% methanol in dichloromethane, collecting 50 mL fractions. The combined fraction 25-30 was purified further on a 1cm column collecting 2 mL fractions using 8% methanol in dichloromethane. Fractions 36 to 40 contained two compounds and were separated further using the same solvent. Fraction 16 contained 6,7-dihydroxylupanine (**A-3**) (23.6 mg). Fractions 32-33 were purified using 10% methanol in dichloromethane, where fraction 37 afforded 7-hydroxylupanine (**A-2**) (52.3 mg). Purification of fractions 51-77 with 15% methanol in dichloromethane resulted in *N*-methylenehydroxycytisine (**A-1**) (40.1 mg) being isolated.

Isolation of compounds from the stem and bark

The dichloromethane extract of the stem and bark (2.40 g) was separated on a 3 cm column sequentially using 500 mL each of a dichloromethane: methanol step gradient with 100% dichloromethane, and then 4%, 8%, 12%, and 15% methanol in dichloromethane. A total of 50×50 mL fractions were collected with ten fractions being collected for each stage. Fractions 12-14 were combined and purified with 1% methanol in dichloromethane to produce methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate (**A-8**) (22.5 mg) in fraction 1 and velutinine (**A-5**) (20.4 mg) in fraction 2. Although the hexane, ethyl acetate and methanol

extracts were also separated into various fractions, no compounds of interest were found in them.

Isolation of compounds from the leaves

The hexane extract of the leaves (48.2 g) was separated successively with 100% hexane and then 10%, 20%, 30%, 40%, 50%, 60% dichloromethane in hexane and 100% dichloromethane with 24 fractions being collected in each stage. Fraction 68 was purified further with 20% dichloromethane in hexane to produce 12-oleanen-3-one (**A-10**) (32.6 mg) in fraction 2. The dichloromethane extract (9.3 g) was separated with 100% dichloromethane and then 5%, 15%, 16%, 20% methanol in dichloromethane, collecting 7 fractions of 50 mL each in each stage from a 3 cm diameter column. Fractions 30-33 was separated with 16% methanol in dichloromethane, where cytisine (**A-7**) (26.0 mg) was obtained in fraction 12. The ethyl acetate and methanol extracts did not contain any compounds of interest.

N-Methylenehydroxycytisine (**A-1**)

Dark brown solid; m.p. 142-145 ^{o}C; $[\alpha]_{D}^{20}$ -320.30° (*c* 0.00638, CH_3OH); UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε) 232 (4.81), 317 (4.94); IR cm^{-1} 3424, 2931, 2855, 2784, 1654, 1560, 1549, 1141, 798, 734; ^{1}H NMR (400 MHz, $CDCl_3$) 7.22 (dd, *J* = 6.8, 9.0 Hz, H-4), 6.44 (d, *J* = 9.0 Hz, H-3), 5.63 (d, *J* = 6.8 Hz, H-5), 4.00 (d, *J* = 15.4 Hz, H-10eq), 3.82 (dd, *J* = 15.4 Hz, 6.6 Hz, H-10ax), 2.74 (H-11eq*), 2.71 (brs, H-7), 2.70 (s, 2H-14), 2.60 (d, *J* = 11.0 Hz, H-13eq), 2.37 (brs, H-9), 2.25 (d, *J* = 9.8 Hz, H-11ax), 1.90 (d, *J* = 1.5 Hz, H-13ax), 1.81 (d, *J* = 14.3 Hz, H-8 eq), 1.67 (d, *J* = 12.8 Hz, H-8ax); ^{13}C NMR (100 MHz, $CDCl_3$) 163.5 (C=O), 151.3 (C-6), 138.6 (C-4), 116.3 (C-3), 105.0 (C-5), 79.7 (C-14), 58.7 (C-11), 57.7 (C-13), 50.0 (C-10), 34.7 (C-7), 27.9 (C-9), 26.2 (C-8). EIMS** *m/z* (rel. int.): 190 (66), 160 (24), 148 (30), 147 (74), 146 (100), 134 (22), 109 (14)

*Multiplicity obscure because of overlap with other resonances.

** The molecular ion at *m/z* 220 could not be detected in the mass spectrum. It is postulated that *N*-methylenehydroxycytisine is unstable and reverts to cytisine during fragmentation.

7-Hydroxylupanine (**A-2**)

Needle like white crystals; m.p. 197-199 °C; $[\alpha]_D^{20}$ +22.32° (*c* 0.0224, CH_2Cl_2); UV λ_{max} (CH_2Cl_2) nm (log ε): 230 (7.57); IR cm^{-1} 3427, 2931, 2857, 2761, 1672, 1352, 1168, 1121, 1055; ^1H NMR (400 MHz, $CDCl_3$): δ 3.65-3.76 (3H, m, 2H-10, H-6), 2.92 (1H, dd, *J* = 13.18, 2.57 Hz, H-17eq), 2.73 (1H, d, *J* = 11.35 Hz, H-15eq), 2.61 (4H, t, *J* = 6.51 Hz, 2H-3/2H-5), 2.50 (1H, brs, OH), 1.96-2.07 (3H, m, H-12, H-13eq, H-15ax), 1.84-1.92 (3H, m, 2H-4, H-17ax), 1.60 (1H, m, H-9), 1.55 (1H, m, H-14eq), 1.53 (1H, m, H-11), 1.42 (1H, m, H-14ax), 1.38 (1H, m, H-8eq), 1.30 (1H, m, H-13ax), 1.18 (1H, m, H-12ax), 0.95 (1H, m, H-8ax); For ^{13}C NMR data see Table 6; MS (EI, 70 eV): *m/z* (%) = 264 $[M]^+$ (20), 222 (12), 152 (43), 138 (100), 110 (39), 97 (36), 83 (39).

6,7-Dihydroxylupanine (**A-3**)

Brown oil; $[\alpha]_D^{20}$ +41.67° (*c* 0.00841, $CHCl_3$); UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε) 228 (5.06), 286 (5.16), 312 (5.20); IR cm^{-1} 3378, 2930, 2856, 1677, 1353, 1170, 1135, 1117; ^1H NMR (400 MHz, $CDCl_3$) δ 3.77 (dd, *J* = 12.8, 2.4 Hz, H-10eq), 3.67 (dd, *J* = 12.8, 9.2 Hz, H-10ax), 2.90 (d, *J* = 12.0 Hz, H-17eq), 2.83 (d, *J* = 11.6 Hz, H-15eq), 2.62 (4H, m, 2H-3, 2H-5), 2.13 (d, *J* = 14.0 Hz, H-17ax), 2.06 (d, *J* = 12.0 Hz, H-15ax), 1.97 (d, *J* = 12.8 Hz, H-12eq), 1.88 (m, 2H-4), 1.78 (d, *J* = 10.6 Hz, H-9), 1.70 (d, *J* = 15.9 Hz, H-11), 1.68 (m, H-14eq), 1.65 (m, H-13eq), 1.60 (brs, H14ax), 1.45 (d, *J* = 13.6 Hz, H-8eq), 1.39 (d, *J* = 12.6 Hz, H-12ax), 1.28 (m, H-13ax), 0.98 (m, H-8ax); For ^{13}C NMR data see Table 6; EIMS *m/z* (rel. int.): 280 $[M]^+$(20),

154 (100), 126 (34), 96 (32), 55 (24); HREIMS 280.1748 $[M]^+$ (280.1787 calculated for $C_{15}H_{24}N_2O_3$).

17-Oxo-thermopsine **(A-4**)

Brown solid; m.p. 215-217 °C; $[\alpha]_D^{20}$ -83.33° (c 0.0012, CH_2Cl_2); UV λ_{max} (CH_2Cl_2) nm (log ε): 228 (6.96), 315 (7.28); IR cm^{-1} 2926, 2856, 2360, 1655, 1544, 1444, 1260; ^1H NMR (400 MHz, $CDCl_3$): δ 7.26 (1H, dd, *J* = 8.97, 6.78 Hz, H-4), 6.46 (1H, dd, *J* = 8.97, 1.28 Hz, H-3), 6.26 (1H, dd, *J* = 6.78, 1.28 Hz, H-5), 4.56 (1H, dd, *J* = 11.35, 2.20 Hz, H-15eq), 4.22 (1H, d, *J* = 15.75 Hz, H-10eq), 3.91 (1H, dd, *J* = 15.75, 6.41 Hz, H-10ax), 3.61 (1H, d, *J* = 2.56 Hz, H-7), 3.32 (1H, d, *J* = 8.79 Hz, H-11), 2.43 (1H, m, H-9), 2.39 (1H, dd, *J* = 13.00, 2.75 Hz, H-15ax), 2.32 (1H, d, *J* = 13.55 Hz, H-8eq), 1.99 (1H, dd, *J* = 13.55, 3.13 Hz, H-8ax), 1.96 (m, H-13eq), 1.60 (5H, m, 2H-12, H-13, 2H-14); For ^{13}C NMR data see Table 6; MS (EI, 70 eV): *m/z* (%) = 258 $[M]^+$ (66), 147 (64), 146 (100), 112 (71), 84 (43).

Velutinine (**A-5**)

Dark brown solid; m.p. 105-107 ^oC; $[\alpha]_D^{20}$ -2.91 (*c* 0.00852, CH_2Cl_2); UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε) 227 (6.19), 286 (6.29), 312 (6.33); IR cm^{-1} 3423, 2926, 1618, 1508, 1498, 1474, 1342, 1289, 1118, 1034, 836; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.4 Hz, H-4), 6.70 (s, H-12), 6.53 (dd, *J* = 8.4, 2.5 Hz, H-3), 6.41 (s, H-16), 6.39 (d, *J* = 2.5 Hz, H-5), 5.90 (d, *J* = 1.5 Hz, H-17a), 5.87 (d, *J* = 1.5 Hz, H-17b), 5.45 (d, *J* = 7.0 Hz, H-8), 4.93 (s, OH), 4.20 (dd, *J* = 11.0, 5.0 Hz, H-10eq), 3.59 (t, *J* = 11.0 Hz, $H\text{-}10_{ax}$), 3.47 (m, H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0 (C-2), 156.6 (C-6), 154.2 (C-11), 148.1 (C-14), 141.7 (C-13), 132.1 (C-4), 117.8 (C-7), 109.7 (C-3), 104.7 (C-12), 103.6 (C-5), 101.3 (C-17), 93.8 (C-16), 78.4 (C-8), 66.4 (C-10), 40.1 (C-9); EIMS *m/z* (rel. int.): 284 $[M]^+$(6), 270 (100), 255 (29), 207 (21), 161 (9), 148 (15), 135 (9); HREIMS 284.0679 $[M]^+$ (284.0797 calculated for $C_{15}H_{12}N_2O_4$).

Antibacterial assay

The bacterial strains used were the Gram-negative *Pseudomonas aeruginosa* (ATCC25922) and the Gram-positive *Enterococcus faecalis* (ATCC29212). Both organisms were maintained in Muller Hinton (MH) Broth overnight.

The samples were dissolved in acetone to a known concentration (1.0 mg mL^{-1}) prior to testing, except for **A-10** and **A-8** which were prepared at 0.7 mg mL^{-1} and 0.8 mg mL^{-1}, respectively. The antibacterial assays followed the format of the serial microdilution assay of Eloff (1998). Two-fold serial dilutions of the samples (100 μL) were prepared in wells of 96-well microtitre plates. Bacterial cells (100 μL of an overnight culture) was then added to each well before incubation for 24 hours at 37 °C. Iodonitrotetrazolium chloride (INT, Sigma, 40 μL of a 0.2 mg mL^{-1} solution) was added to each well as an indicator of bacterial growth. INT, a colourless tetrazolium salt is converted to a red-coloured formazan product by actively dividing cells. The minimum inhibitory concentration (MIC) was visually read as the lowest concentration of sample that inhibited microbial growth, as indicated by a visible reduction in the red colour of the INT formazan. In each assay a negative solvent control and a positive control were included. Gentamicin (Sigma) was used as the antibacterial agent. The samples were tested in triplicate.

Acknowledgements

We are grateful to the National Research Foundation (NRF) of South Africa for financial support and a bursary for E. Korir, and to Dr R. Clark of Pretoria for facilitating access to plant materials. The project was funded through the Thuthuka Programme of the NRF.

2.5 References

Abbott, B.J., Hartwell, J.L., Leiter, J., Perdue Jr., R.E., Schepartz, S.A. 1966. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. 38. Plant extracts. Cancer Research. 26, 1461-1656.

Abdel-Halim, O.B., 1995. (-)-6α-Hydroxylupanine, a lupin alkaloid from *Lygos raetam* var. *sarcocarpa*. Phytochemistry. 40, 1323-1325.

Aniszewski, T., 2007. Alkaloids - Secrets of Life Alkaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier. Kidlington, the Netherlands, pp 98-101.

Asres, K., Gibbons, W.A., Philipson, J.D., Mascagni, P., 1986. The alkaloids of *Sophora velutina*. Journal of Natural Products. 49, 117-121.

Brummitt, R.K. and Gillett, J.B., 1966. Notes on the genus *Sophora* in Africa, including an Asian species found near Zimbabwe. Kirkia. 5, 259-270.

Bruneton, J., 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. Intercept, Andover, UK.

Chang, H.M., But, P.P.H., 1986. Pharmacology and Applications of Chinese Materia Medica. World Scientific Publishers, Singapore, pp. 736.

Chiu, H.L., Wu, J.H., Tung, Y.T., Lee, T.H., Chien, S.C., Kuo, Y.H., 2008. Triterpenoids and aromatics from *Derris laxiflora*. Journal of Natural Products. 71, 1829-1832.

Dewick, P.M., 1994. Isoflavonoids, in: Harborne, J.B., (Ed.), The Flavonoids. Advances in Research since 1986. Chapman and Hall Ltd., New York, pp. 123.

Eloff, J.N., 1998. A sensitive and quick method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica. 64, 711-713.

Gelfand, M., Mavi, S., Drummond, R.B., Ndemera, B., 1985. The Traditional Medical Practitioner in Zimbabwe. Mambo Press, Gweru.

Hoffmann, D., 2003. Medical Herbalism. The Science and Practice of Herbal Medicine. Healing Art press, Vermont, pp. 130-133.

Huang, K.C., 1993. The Pharmacology of Chinese Herbs. CRC Press Inc., Boca Raton, FL., pp. 63-66.

Kinghorn, A.D., Balandrin, M.F., 1984. Quinolizidine alkaloids of the Leguminosae: Structural types, analysis, chemotaxonomy and biological properties in: Alkaloids: Chemical and Biological Perspectives. Volume 2, Pelletier, S.W. (Ed.), Elsevier Science, Oxford, UK, 105-149.

Kite, G.C., Pennington, R.T., 2003. Quinolizidine alkaloid status of *Styphnolobium* and *Cladrastis* (Leguminosae). Biochemical Systematics and Ecology. 31, 1409-1416.

Koorbanally, N.A., 1997. Extractives from the Amaryllidacea and Fabaceae, MSc dissertation, University of Natal (now UKZN), South Africa.

Liu, J., Zhu, M., Shi, R., Yang, M., 2003. *Radix sophorae flavescentis* for chronic hepatitis B: a systematic review of randomized trials. American Journal of Chinese Medicine. 31, 337-354.

Ma, S.C., Du, J., But, P.P.H., Deng, X.L., Zhang, Y.W., Ooi, V.E.C., Xu, H.X., Lee, S.H.S., Lee, S.F., 2002. Antiviral Chinese medicinal herbs against respiratory syncytial virus. Journal of Ethnopharmacology. 79, 205-211.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids. A compilation and some salient features. Phytochemistry. 37, 1517-1575.

Mikhova, B., Duddeck, H., 1998. ^{13}C NMR spectroscopy of tri- and tetracyclic quinolizidine alkaloids. Compilation and discussion. Magnetic Resonance in Chemistry. 36, 779-796.

Ohmiya , S., Saito, K., Murakoshi, I., 1988. Lupine alkaloids, in: Cordell, G. (Eds.), The Alkaloids. Academic Press, London, Vol 47, pp. 2-105.

Pennington, R.T., Stirton, C.H., Schrire, B.D., 2005. Tribe Sophoreae. in: Lewis, G., Schrire, B., Mackinder, B., Lock, M. (Eds.), Legumes of the World. Royal Botanic Gardens, Kew, pp 227-243.

State Pharmacopoeia Commission of P.R.C., 2000. Pharmacopoeia of the People's Republic of China. Chemical Industry Press, Beijing. 1, 197.

Tang, W., Eisenbrand, G., 1992. Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. Springer-Verlag, Berlin, pp. 931-943.

Wang, Y.H., Li, J.S., Jiang, Z.R., Kubo, H., Higashiyama, K., Ohimiya, S., 2000. Lupine alkaloids from Chinese *Maackia amurensis*. Chemical and Pharmaceutical Bulletin (Japan). 48, 641-645.

Wiewiorowski, M., Edwards, O.E., Bratek-Wiewiorowska, M.D., 1967. Conformation of the C_{15} lupine alkaloids. Canadian Journal of Chemistry. 45, 1447-1457.

Zhu, Y.P., 1998. Chinese Materia Medica: Chemistry, Pharmacology and Applications. Harwood Academic Publishers, Amsterdam, pp. 149.

Chapter 3. Isoflavones from *Calpurnea aurea* subsp. *aurea* and their anticancer activity

Erick Korir[1], Joyce J. Kiplimo[1], Neil R. Crouch[1,2], Nivan Moodley[3] and Neil. A. Koorbanally[1*]

[1]School of Chemistry and Physics, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa
[2]Ethnobotany Unit, South African National Biodiversity Institute, PO Box 52099, Berea Road, 4007, Durban, South Africa
[3]Defence, Peace, Safety and Security (DPSS), Centre for Scientific and Industrial Research (CSIR), Pretoria, South Africa

*corresponding author: Koorbanally@ukzn.ac.za; Tel +2731 260 3189; Fax +2731 260 3091

Abstract

The isoflavones, 4′,5,7-trihydroxyisoflavone (**B-1**), 7,3′-dihydroxy-5′-methoxyisoflavone (**B-2**), 7-hydroxy-4′,8-dimethoxyisoflavone (**B-3**), 7-acetoxy-4′,8-dimethoxyisoflavone (**B-4**) and 3',7-dihydroxy-4′,8-dimethoxyisoflavone (**B-5**), a pterocarpan (3-acetoxy-9-methoxypterocarpan) (**B-6**) and a quinolizidine alkaloid (calpurnine) (**B-7**) were isolated from the stem and bark of *Calpurnia aurea*. These isoflavones were screened for *in vitro* anticancer activity against breast (MCF7), renal (TK10) and melanoma (UACC62) human cell lines, where **B-5**, with an added hydroxyl group on the phenyl ring was found to be the most active amongst all the compounds tested, followed by **B-2**, also with a hydroxyl and methoxy group on the phenyl ring but in the 3' and 5' positions and not the 3' and 4' positions as in **B-5**.

Keywords: *Calpurnia aurea*, Fabaceae, 5,6′-dihydroxy-2′,6-dimethoxyisoflavone, anti cancer.

3.1 Introduction

Calpurnia aurea (Ait.) Benth. is a shrub to slender tree of up to 15 m tall, widespread along the east coast of Africa, throughout which range it is used in traditional medicine and for various utilitarian purposes. The genus *Calpurnia* E. Mey. is currently assigned to the tribe Podalyrieae of the Papilionaceae subfamily (van Wyk, 2005). It was initially considered to belong to the *Sophora* group of the primitive tribe Sophoreae sensu Polhill (1981) but later studies transferred *Calpurnia* to the tribe Poldalyrieae (Polhill *et al*., 1994; van Wyk and Schutte, 1995).

It is used by the Shinasha people of Northern Ethiopia to treat amoebiasis and giardiasis while the Amhara people from the same region use the leaves to treat malaria and the seeds to treat hypertension while a combination of the leaves and seeds are used to treat diarrhoea, rabies and diabetes (Giday *et al*., 2007). The plant has also been used as an insecticide to kill lice (Palmer and Pitman, 1972; Waka *et al*., 2004), to induce uterine contractions (Desta *et al*. 1994), and to treat coughs, amoebic dysentery, syphilis, leishmaniasis, tapeworm, trachoma, ringworm, scabies, elephantiasis, abscesses and wounds as well as stomach ache, vomiting, headache and eye diseases (Jansen 1981; Abebe, 1986; Asres *et al*., 2001; Tadeg *et al*., 2005; Teklehaymanot and Giday, 2007). Both in East and southern Africa, plant extracts are employed in treating wounds infested with maggots (Palmer and Pitman 1972; Kokwaro, 1976), to the extent that its Zulu name is umKhiphampethu, meaning “maggot-extracter”. Its widespread application for diverse ethnomedicinal uses has made it a subject for many pharmacological (e.g. Desta *et al*., 1994) and phytochemical studies.

Pharmocological studies have shown that the methanol extracts of the leaves and stems of *C. aurea* have good antibacterial and antioxidant properties (Tadeg *et al*., 2005; Adedapo *et al*.,

2008), validating its traditional use for a range of microbial infections. Insecticidal activity was also shown by the methanol and water extracts against the rice weevil (*Sitophilus oryzae*) (Louis *et al*., 2007), in keeping with its ethnobotanical use against lice and maggots. The oil extract of the dried leaves was observed to attract and be toxic to two species of ticks, *Rhipicephalus pulchellus* and *Rhipicephalus appendiculatus*, revealing a potential application as an acaricidal trap bait (Nana *et al*., 2010; Zorloni *et al*., 2010).

There are seven *Calpurnia* species (eight taxa) (Beaumont *et al*., 1999) of which only one, *C. aurea* (syn. *Calpurnia subdecandra* (L'Hérit.) Schweick.) has been investigated for its phytochemical constituents. A literature survey on the species *C. aurea* shows several previous investigations of the plant under subspecies *aurea* and *sylvatica*. However, *C. aurea* subsp. *sylvatica* (Burch.) Brummitt is no longer considered distinct from *C. aurea* subsp. *aurea* and has accordingly been synonymised (Beaumont *et al*., 1999). The Indian endemic *C. aurea* subsp. *indica* Brummitt, is though, still recognised. Two early phytochemical studies of C. *aurea* subsp. *aurea* reported the isolation of agglutinins from the seeds to antigens A and B of human erythrocytes (Bird, 1957; Potapov, 1968), whilst a third (as syn. *C. subdecandra*) yielded the novel quinolizidine alkaloid, calpurnine (Goosen, 1963). Subsequent investigations reported several more quinolizidine alkaloids, characteristic chemotaxonomic markers for the Fabaceae.

The quinolizidine alkaloids 13-hydroxylupanine and its angelate and tiglate esters and virgiline and its pyrrolecarboxylic acid ester were first isolated from the leaves and twigs of the Ethiopian *C. aurea* subsp. *aurea*, along with the previously reported calpurnine (van Eijk and Radema, 1977). Shortly thereafter, these same compounds as well as calpurmenine and its 13-pyrrolylcarboxyl ester were found in South African material of *C. aurea* (as subsp.

sylvatica) (Radema *et al*., 1979). A subsequent reinvestigation of the leaves of Ethiopian *C. aurea* subsp. *aurea* (Asres *et al*., 1986a; 1986b) revealed the presence of calpurmenine and its 13-pyrrolylcarboxyl ester, resulting in a total of seven compounds common to both South African and the Ethiopian chemotypes as well as an additional six alkaloids, epilupanine, lupinine, 3β,4α,13α-trihydroxylupanine, 3β,4α-dihydroxy-13-*O*-(2'-pyrrolylcarbonyl)-lupanine (calpaurine), 4β-hydroxy-13α-*O*-(2'-pyrrolylcarbonyl)-lupanine (digittine) and 4β,13α-dihydroxylupanine. Along with the previously isolated *O*-(2-pyrrolylcarbonyl) virgiline, the 2,3-dehydro-*O*-(2-pyrrolylcarbonyl) virgiline was also isolated (Kubo *et al*., 1984), bringing the total number of quinolizidine alkaloids isolated from *C. aurea* subsp. *aurea* to 15.

Apart from the quinolizidine alkaloids, the flavonoids vicenin-2 (6,8-di-β-D-glucopyranosyl-5,7,4'-trihydroxyflavone), butin (7,3',4'-trihydroxyflavanone) and 3'-hydroxydaidzein (7,3',4'-trihydroxyisoflavone) were isolated from the seeds of *C. aurea*, in keeping with flavonoids being the other major class of compounds consistently found in the Fabaceae (de Nysschen *et al*., 1998).

Since there have no previous reports on the wood and stem bark of *C. aurea*, we have carried out a phytochemical analysis of these components to enable a more complete phytochemical analysis of this species. We report herein the isolation of five isoflavonoids, a pterocarpan and a quinolizidine alkaloid from the stem and bark of *C. aurea* as well as the anticancer activity of the isolated isoflavonoids. Isoflavones and in particular genistein (5,7,4'-trihydroxyisoflavone) are known to possess antitumor effects (Barnes, 1997) by preventing the formation of hormone induced breast cancer (Bruneton, 1995). Since the isoflavones here

isolated from *C. aurea* were all substituted at the 7 and 4' positions, they were ideal candidates for the evaluation of their anticancer activity.

3.2 Results and Discussion

The stem and bark hexane extract yielded the widely studied genistein (4',5,7-trihydroxyisoflavone) (**B-1**) (Wang *et al*., 1999; Dixon and Ferreira, 2002), 5',7-dihydroxy-3'-methoxyisoflavone (**B-2**) (An *et al*., 2008; Li *et al*., 2009), 7-hydroxy-4',8-dimethoxyisoflavone (8-O-methylretusin; isoafrormosin) (**B-3**) (Jurd *et al*., 1972; Hayashi and Thomson, 1974; Harper *et al*., 1976; Chen *et al*., 1983), 7-acetoxy-4',8-dimethoxyisoflavone (**B-4**) and 3',7-dihydroxy-4',8-dimethoxyisoflavone (**B-5**) (Harper *et al*., 1976; de Oliveira *et al*., 1978; Albuquerque *et al*., 1981), along with a pterocarpan, 3-acetoxy-9-methoxypterocarpan (**B-6**) (Al-Ani *et al*., 1984) and a quinolizidine alkaloid calpurnine (**B-7**) (Asres, *et al*., 1986a) (Figure 3).

To our knowledge, this is the first report of **B-4** from a plant source. Other reports contain information on the tri-acetylated 7-hydroxy-4',8-dimethoxyisoflavone (Jurd *et al*., 1972; Hayashi and Thomson, 1974; Harper *et al*., 1976; Chen *et al*., 1983). The NMR data reported in Hayashi and Thomson (1974) for both compounds **B-3** and **B-4** are erroneous in that the assignments of the two methoxy resonances must be interchanged (8-OCH_3 should be at δ_H 4.06 and 4'-OCH_3 at δ_H 3.90), since our NOESY data shows that the 4'-methoxy resonance shows a NOESY correlation to the H-3'/5' resonance at δ_H 6.90.

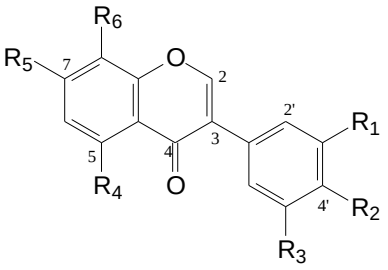
The five isolated isoflavones were either tri- or tetra-substituted at positions 5, 7 and 8 on the A ring and 3', 4' or 5' on the phenyl ring (ring C). Biosynthetically, substitution at the 5 and 7 positions occur readily because of the polyketide pathway, however species within the

Sophoreae have also been popularly substituted at the 7 and 8 positions as well as at the 3' and 4' positions on the phenyl ring (Harper *et al*., 1976; Albuquerque *et al*., 1981; Bezuidenhout *et al*., 1988), consistent with the isoflavones isolated from *C. aurea* in this work. It is highly likely that the isoflavones **B-3-B-5** follow the same biosynthetic pathway and most probable that **B-1** and **B-2** is also linked to this pathway prior to dehydroxylations and demethoxylations taking place en route to **B-3-B-5**. The other isoflavonoid isolated from the seeds, 3'-hydroxydaidzen (de Nysschen *et al*., 1998), is also hydroxylated at the 7, 3' and 4' positions. Furthermore, the isolation of **B-1, B-3** and **B-5** from *Monopteryx inpae* W.A. Rodrigues (Albuquerque *et al*., 1981) and **B-3** and **B-5** from *Xanthocercis zambesiaca* (Baker) Dumaz-le-Grand (Harper *et al*., 1976) of the Sophoreae, demonstrate the relatively close relationship of the tribes Podalyrieae and Sophoreae within the subfamliy Papilionoideae.

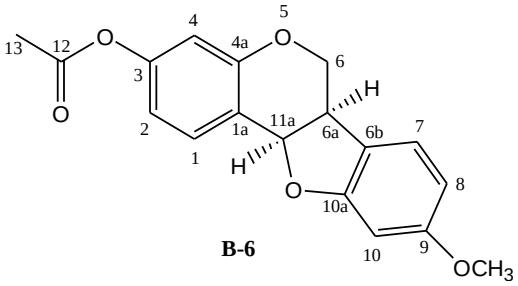
The anticancer activity of the isoflavonoids **B-2-B-5** are shown in Table 8 in the form of the response parameters GI_{50}, Total growth inhibition (TGI) and LC_{50}, which are interpolated values from the concentration response curves where the net percentage growth is plotted against the concentration of each compound and represents the concentrations of the compounds in μg/mL at which the net percentage growth is +50, 0 and -50, respectively. Due to insufficient amounts isolated, genistein (**B-1**) was not subject to the anticancer screening.

All the tested compounds exhibited concentration-dependendent inhibition up to 100 μg/mL, with compounds **B-2**, **B-3** and **B-5** being most active against the melanoma (UACC-62) cell line with GI_{50} values of 31.01, 40.14 and 27.35 μg/mL (Table 8). Compounds **B-2** and **B-5** were also active against the breast (MCF-7) cell line with GI_{50} values of 45.51 and 31.92 μg/mL. From all the compounds tested, compound **B-5** seemed to have the best overall

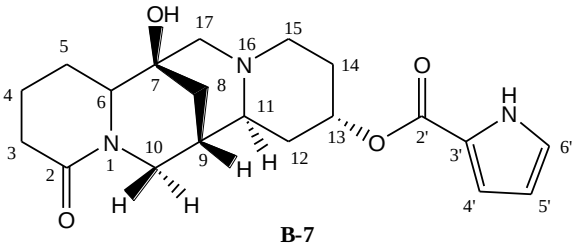
activity in all three cell lines having the lowest GI_{50} in each at 45.81, 27.35 and 31.92 μg/mL and was the only compound of those tested to show TGI for all three cancer cell lines at below 100 μg/mL and a TGI for the melanoma (UACC-62) cell line of 52.49 μg/mL.



	R_1	R_2	R_3	R_4	R_5	R_6
B-1		OH		OH	OH	
B-2	OH		OCH_3		OH	
B-3		OCH_3			OH	OCH_3
B-4		OCH_3			$OC(O)CH_3$	OCH_3
B-5	OH	OCH_3			OH	OCH_3



B-6



B-7

Figure 3 Compounds isolated from *Calpurnia aurea*

Structurally, **B-3**-**B-5** are all methoxylated at both the 8 and 4' positions with **B-4** being acetylated at C-7 whereas the others are hydroxylated at C-7. **B-5** also contains an extra hydroxyl group at the 3' position on the phenyl ring and this added hydroxyl group resulted in improved anticancer activity. Acetylation at C-7 however led to a loss of activity as seen in

B-4, which was the most inactive of all the compounds tested. **B-2** had a unique substitution pattern to the other three compounds tested, in that it was the only isoflavone not to be methoxylated at C-8 and C-4'. Nevertheless, hydroxylation at C-7 and methoxylation and hydroxylation at each of the *meta* positions on the phenyl ring resulted in better activity than all the other isoflavones tested with the exception of **B-5**.

Table 8 Growth Inhibition values for compounds **B-2-B-5** against TK-10, UACC-62 and MCF-7 cell lines.

Compound	**concentration-response parameters**	**Line 1 (TK-10) Renal**	**Line 2 (UACC-62) Melanoma**	**Line 1 (MCF-7) Breast**
B-2	GI_{50}	50.57	31.01	45.51
	TGI	N/A	60.28	78.40
	LC_{50}	N/A	89.55	N/A
B-3	GI_{50}	55.44	40.14	69.05
	TGI	N/A	90.41	N/A
	LC_{50}	N/A	N/A	N/A
B-4	GI_{50}	69.89	53.34	66.19
	TGI	N/A	N/A	N/A
	LC_{50}	N/A	N/A	N/A
B-5	GI_{50}	45.81	27.35	31.92
	TGI	91.06	52.49	57.22
	LC_{50}	N/A	77.62	82.52
Etoposide	GI_{50}	4.88	0.74	0.57
	TGI	36.77	16.41	N/A
	LC_{50}	85.38	84.58	N/A

It is reported that isoflavones from soybean also have preventive anticancer activity and that the methylated isoflavones (glycitein, biochanin A and formononetin) have much greater anticancer activity than those without methyl groups (Walle *et al*., 2007). Studies suggest that

isoflavones with methoxy groups appear to have more beneficial qualities than their non-methylated counterparts and have been shown to be more bioavailable and biologically stable than the hydroxylated isoflavones (Wen *et al*., 2006). This may account for the anticancer activity shown by the isolates of *C. aurea* in this work as all the tested compounds were methoxylated at some point on the skeletal framework.

3.3 Experimental

General experiment procedures: The melting points were recorded on an Ernst Leitz Wetzler micro-hot stage melting point apparatus. UV spectra were recorded on a Varian Cary UV-VIS spectrophotometer and IR spectra were recorded on a Perkin-Elmer Universal ATR spectrometer. The ^{1}H, ^{13}C and all 2D NMR spectra were recorded using a Bruker Avance^{III} 400 MHz spectrometer at room temperature using either deuterated methanol (CD_3OD) or deuterated chloroform ($CDCl_3$) as solvent. Specific rotations were measured at room temperature in methanol on a PerkinElmerTM, Model 341 polarimeter with a 10 mm flow tube. For GC-MS analyses, the samples were analysed on an Agilent GC–MSD apparatus equipped with DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column. Helium (at 2 ml/min) was used as a carrier gas. The MS was operated in the EI mode at 70 eV. The separation, isolation and purification of compounds were carried out by gravity column chromatography using Merck silica gel 60 (0.040-0.063 mm) and monitored by thin layer chromatography (TLC; Merck 20 × 20 cm silica gel 60 F_{254} aluminum sheets).

Plant collection and extraction

The stem and bark of *Calpurnia aurea* (Ait.) Benth. was obtained from a cultivated specimen in Kloof, Durban. A voucher specimen (*N. Crouch 1279*, NH) was deposited at the

KwaZulu-Natal Herbarium, Durban, South Africa for verification purposes. The stem and bark was milled and then extracted separately using a soxhlet apparatus with hexane, dichloromethane, ethyl acetate and methanol successively for 24 hours each. The dry milled stem and bark (651.8 g mass) yielded 3.2 g, 3.4 g, 10.7 g, and 72.1 g extracts for each of the four solvents mentioned above.

Separation and purification

The hexane extract of the stem and bark (3.2 g) was separated successively with 100% hexane and then a hexane : dichloromethane step gradient (10% increments up until 100% dichloromethane), with 20 fractions of 100 mL being collected in each stage off a 4 cm diameter column. Further purifications were carried out in 1 cm diameter columns collecting 5 mL fractions. Fraction 10 was purified further with 15% dichloromethane in hexane to produce 7-acetoxy-4′,8-dimethoxyisoflavone (40.1 mg) (**B-4**) in fraction 21-22. Fraction 42 was purified further using the same solvent system to afford 3-acetoxy-9-methoxypterocarpan (42.7 mg) in fractions 7-9.

The dichloromethane extract of the stem and bark (3.40 g) was separated on a 3 cm diameter column sequentially using 1 L each of a dichloromethane:methanol step gradient with 100% dichloromethane, and then 2%, 4%, 6% and 8% methanol in dichloromethane. A total of 50 × 100 mL fractions were collected with ten fractions being collected for each stage. Subsequent purifications were carried out on 1 cm diameter columns collecting 5 ml fractions. Fraction 8 was purified with 1% methanol in dichloromethane, where fraction 3 was further purified with the same solvent system to afford 7-hydroxy-4′,8-dimethoxyisoflavone (**B-3**) (49.6 mg) in fractions 14-17. Fraction 32 of the crude column was also purified with 1% methanol in dichloromethane to produce 3',7-dihydroxy-4',8-dimethoxyisoflavone (**B-5**) (46.7 mg) in

fraction 40-48. Fractions 41-50 of the crude column were combined and purified further with 1% methanol in dichloromethane to produce 3',7-dihydroxy-5'-methoxyisoflavone (**B-2**) (37.4 mg) in fraction 3-7.

TLC analysis of ethyl acetate and methanol extracts had similar components and these extracts were combined and separated with a dichloromethane: ethyl acetate step gradient of 100:0, 90:10, 80:20, 60:40, 40:60, 0:100 in a 3 cm column with a total of 120 fractions being collected (20 x 50 ml fractions for each gradient). Purifications were carried out on 1 cm diameter columns collecting 5 ml fractions. Fractions 12-15 were combined and purified further with 2% methanol in dichloromethane to afford genistein (4',5,7-trihydroxyisoflavone) (**B-1**) (48.9 mg) in fractions 12-15. Fractions 8-10 were combined and purified with 2% methanol in dichloromethane to afford calpurnine (**B-7**) (39.8 mg) in fractions 69-74.

Compounds **B-1-B-7** were identified from their ^{1}H and ^{13}C NMR, IR, UV and MS data as well as their physical characteristics and melting points and verified by comparing the data to those found in the literature.

Anticancer activity

Anticancer screening was carried out using a method developed by the national cancer institute and transferred to the CSIR (South Africa) in 1999 and known as the three cell prescreening method (Fouche *et al*., 2006; 2008). Breast (MCF-7), renal (TK-7) and melanoma (UACC-62) cell lines were chosen due to their high sensitivity to detect anticancer activity (Fouche *et al*., 2008). The three cell lines were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) medium containing 5% fetal bovine serum and 2 μM L-glutamine. The cells were then inoculated into 96-well microtiter plates with densities

ranging between 5,000 and 40,000 cells per well. A volume of 100 µL of the medium was introduced into the microtiter plates and subsequently incubated at 37^{o}C in a 5:95 (carbon dioxide: air) atmosphere with 100% relative humidity for 24 hours.

The test compounds were dissolved in dimethyl suphoxide (DMSO) and added to the cells at concentrations ranging between 0.001 µg/mL and 100 µg/mL. The cells were then incubated for 48 hours at 37 °C in a humidified atmosphere, followed by the fixing of the cells *in situ* with trichloroacetic acid (TCA) and staining with 100 µL sulforhodamine B (SRB) solution. Unbound dye was removed by washing with 1% acetic acid and air drying the plates. Bound stain was solubilized with 10 µM trizma base and the optical density was read on an automated plate reader at a wavelength of 540 nm.

The percentage growth of human tumor cells was determined spectrometrically by measuring the difference in optical density of the control (C) at the start (T_0) and end of drug exposure (T). If $T \geq T_o$ either no effect is experienced or inhibition occurs. Inhibition occurs if $T<C$ and no effect is experienced if $T=C$ (Monks *et al*., 1991). The concentration-response parameters, GI_{50} (the concentration at which the growth of the cell is inhibited by 50%) and LC_{50} (the concentration at which 50% of the cells are killed) are calculated using T, T_0 and C where GI_{50} is the concentration at which $(T-T_0)/(C-T_0) = 0.5$ and LC_{50} is calculated as $(T-T_0)/(C-T_0) = -0.5$. The total growth inhibition (TGI) value symbolizes cytostatic activity and refers to the concentration at which total cell growth is inhibited (i.e. $T = T_0$). The calculations of the concentration-response parameters required for plotting the concentration-response curves were performed at the CSIR (Pretoria, South Africa).

3.4 Conclusion

The stem and bark of *C. aurea* was investigated phytochemically for the first time and yielded a quinolizidine alkaloid, calpurnine found in other parts of the plant as well five isoflavones and a pterocarpan. Isoflavones were only found in the seed of *C. aurea* previously and all the isoflavones isolated in this work as well as the pterocarpan were isolated for the first time from this source. These findings show the close chemical relationship between the Podalyrieae and the Sophoreae in that three of the isoflavones were common to both tribes. Furthermore, the isoflavones were shown to have moderate activity against the renal, melanoma and breast cancer cell lines tested against, with the 7-hydroxy-8-methoxy substitution on the chromone ring and 3'-hydroxy-4'-methoxy substitution on the phenyl ring as in compound **B-5** showing the best activity.

Acknowledgements

The authors are grateful to the NRF for a grant holders bursary for funds used throughout the duration of this project.

3.5 References

Abebe, W., 1986. A survey of prescriptions used in traditional medicine in Gonda region, Northwest Ethiopia: General pharmaceutical practice. Journal of Ethnopharmacology. 18, 147-165.

Al-Ani, H.A.M., Dewick, P.M., 1984. Isoflavonoid biosynthesis: concerning the aryl migration. Journal of the Chemical Society, Perkin Transactions 1. 12, 2831-2838.

Albuquerque, F.B., Braz, R.F., Gottlieb, O.R., Magalhaes, M.T., Maia, J.G.S., de Oliveira, A.B., de Oliveira, G.G., Wilberg, V.C., 1981. Isoflavone evolution in *Monopteryx*. Phytochemistry. 20, 235-236.

An, R.B., Jeong, G.S., Kim, Y.C., 2008. Flavonoids from the heartwood of *Dalbergia odorifera* and their protective effect on glutamate-induced oxidative injury in HT22 cells. Chemical Pharmaceutical Bulletin. 56, 1722-1724.

Adedapo, A.A., Jimoh, F.O., Koduru, S., Afolayan, A.J., Masika, P.J., 2008. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. BMC Complementary and Alternative Medicine. 8, 53-61.

Asres, K., Gibbons, W.A., Phillipson, J.D., Mascagni, P., 1986a. Alkaloids of Ethiopian *Calpurnia aurea* Subsp. *aurea*. Phytochemistry, 25, 1443-1447.

Asres, K., Phillipson, J.D., Mascagni, P., 1986b. Two novel minor alkaloids from Ethiopian *Calpurnia aurea* Ssp. *Aurea*. Planta Medica. 302-304.

Asres, K., Bucar, F., Kartnig, T., Witvrouw, M., Pannecouque, C., de Clercq, E., 2001. Antiviral activity against human immunodeficiency virus type 1 (HIV1) and type 2 (HIV 2) of ethnobotanically selected Ethiopian medicinal plants. Phytotherapy Research. 15, 62-69.

Barnes, S., 1997. The chemopreventive properties of soy isoflavonoids in animal models of breast cancer. Breast Cancer Research and Treatment. 46, 169-179.

Beaumont, A.J., Beckett, R.P., Edwards, T.J., Stirton, C.H., 1999. Revision of the genus *Calpurnia* (Sophoreae: Leguminosae). Bothalia. 29, 5-233.

Bezuidenhout, S.C., Bezuidenhout, B.C.B., Ferreira, D., 1988. α-Hydroxydihydrochalcones and related 1,3-diarylpropan-2-ones from *Xanthocercis zambesiaca*. Phytochemistry. 27, 2329-2334.

Bird, G. 1957. Hemagglutinins in *Calpurnia aurea*. Nature. 180, 657.

Bruneton, J., 1995. Pharmacognosy, Phytochemistry, Medicinal plants. Intercept Limited, Andover, UK.

Chen, C.C., Chen, Y.L., Chen, Y.P., Hsu, H.Y., 1983. A study on the constituents of *Millettia reticulata* Benth. Taiwan Yaoxue Zazhi. 35, 89-93.

Desta, B., 1994. Ethiopian traditional herbal drugs. Part III: Anti-fertility activity of 70 medicinal plants. Journal of Ethnopharmacology. 44, 199-209.

Dixon, R.A., Ferreira, D., 2002. Genistein. Phytochemistry. 60, 205-211.

Fouche, G., Cragg, G.M., Pillay, P., Kolesnikova, N., Maharaj, V.J., Senabe, J., 2008. *In vitro* anticancer screening of South African plants, Journal of Ethnopharmacology. 119, 455-461.

Fouche, G., Khorombi, E., Kolesnikova, N., Maharaj, V.J., van der Merwe, M., Nthambeleni, R., 2006. Investigation of South African plants for anticancer activity, Pharmacology. online, 3, 494-500.

Giday, M., Teklehaymanot, T., Mekonnen, Y., 2007. Medicinal plants of the Shinasha, Agewawi and Amhara peoples in northwest Ethiopia. Journal of Ethnopharmacology. 110, 516–525.

Goosen, A., 1963. The alkaloids of the Leguminosae. Part I. The structure of calpurnine from *Calpurnia subdecandra*. Journal of the Chemical Society. 3067-3068.

Harper, S.H., Shirley, D.B., Taylor, D.A., 1976. Isoflavones from *Xanthocercis zambesiaca*. Phytochemistry. 15, 1019-1023.

Hayashi, T., Thomson, R.H., 1974. Isoflavones from *Dipteryx odorata*. Phytochemistry. 13, 1943-1946.

Jansen, P.C.M., 1981. Spices, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance. Agricultural Research Reports 906. Centre for Agricultural Publishing and Documentation, Wageningen.

Jurd, L., Stevens, K., Manners, G., 1972. Isoflavones from the heartwood of *Dalbergia retusa*. Phytochemistry. 11, 2535-2540.

Kokwaro, J.O., 1976. Medicinal plants of East Africa. East African Literature Bureau, Nairobi.

Kubo, I., Matsumoto, T., Kozuka, M., Chapya, A., Naoki, H., 1984. Quinolizidine alkaloids from the African medicinal plant *Calpurnia aurea*: molluscicidal activity and structural study by 2D NMR. Agricultural and Biological Chemistry. 48, 2839-2841.

Li, X., Li, J., Wang, D., Wang, W., Cui, Z., 2009. Chromone and flavonoids from *Maackia amurensis*. Asian Journal of Traditional Medicine. 4, 98-103.

Louis, S., Delobel, B., Gressent, F., Duport, G., Diol, O., Rahioui, I., Charles, H., Rahbé, Y., 2007. Broad screening of the Legume family for variability in seed insecticidal activities and for the occurrence of the A1b-like knottin peptide entomotoxins. Phytochemistry. 68, 521-535.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, M., 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. Journal of the National Cancer Institute. 83, 757-766.

Nana, P., Maniania, N.K., Maranga, R.O., Kutima, H.L., Boga, H.I., Nchu, F., Eloff, J.N., 2010. Attraction response of adult *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus* (Acari: Ixodidae) ticks to extracts from *Calpurnia aurea* (Fabaceae). Veterinary Parasitology. 174, 124-130.

de Nysschen, A-M., van Wyk, B-E., van Heerden, F.R., 1998. Seed flavonoids of the Podalyrieae and Liparieae. Plant Systematics and Evolution. 212, 1-11.

de Oliveira, A.B., Iracema, M., Madruga, L.M., Gottlieb, O.R., 1978. Isoflavonoids from *Myroxylon balsamum*. Phytochemistry. 17, 593-595.

Palmer, E., Pitman, N., 1972. Trees of southern Africa. Volume 2. A. A. Balkema, Cape Town.

Polhill, R.M., 1981. Sophoreae, in: Polhill, R.M., Raven, P.H., (Eds.), Advances in legume systematics, part 1. Royal Botanic Gardens, Kew, pp 213-230.

Polhill, R.M., 1994. Classification of the Leguminosae, in: Bisby, F.A., Buckingham, J., Harborne, J.B., (Eds.), Phytochemical dictionary of the Leguminosae, XXXV–LVII. Chapman and Hall, New York.

Potapov, M.I., 1968. Plant agglutinins to human antigen A. Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya. 1, 59-66.

Radema, M.H., van Eijk, J.L., Vermin, W., de Kok, A.J., Romers, C., 1979. Alkaloids of South African samples of *Calpurnia aurea*. Phytochemistry. 18, 2063-2064.

Tadeg, H., Mohammed, E., Asres, K., Gebre-Mariam, T., 2005. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethnopharmacology. 100, 168-175.

Teklehaymanot, T., Giday, M., 2007. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. Journal of Ethnobiology and Ethnomedicine. 3, 12-23.

van Wyk, B-E., 2005. Tribe Podalyrieae, in: Lewis, G., Schrire, B., MacKinder, B., Lock, M. (Eds.), Legumes of the World. Royal Botanic Gardens, Kew. pp 267-271,

van Wyk, B-E., Schutte, A.L., 1995. Phylogenetic relationships in the tribes Podalyrieae, Liparieae and Crotalarieae, in: Crisp, M.D., Doyle, J.J., (Eds.), Advances in Legume Systematics, part 7: Phylogeny. Royal Botanic Gardens, Kew. pp 283–308.

van Eijk, J.L., Radema, M.H., 1977. Some alkaloids of Ethiopian *Calpurnia aurea* and *Cadia purpurea*. Planta Medica. 32, 275-279.

Waka, E.M., Hopkins, R.J., Curtis, C., 2004. Ethnobotanical survey and testing of plants traditionally used against hematophagous insects in Eritrea. Journal of Ethnopharmacology. 95, 95-101.

Walle, T., Ta, N., Kawamori, T., Wen, X., Tsuji, P.A., Walle, U.K., 2007. Cancer chemopreventive properties of orally bioavailable flavonoids-methylated versus unmethylated flavones. Biochemical Pharmacology. 73, 1288-1296.

Wang, H., Nair, M.G., Strasburg, G.M., Booren, A.M., Gray, J.I., 1999. Antioxidant Polyphenols from Tart Cherries (*Prunus cerasus*). Journal of Agricultural and Food Chemistry. 47, 840-844.

Wen, X., Walle, T., 2006. Methylated flavonoids have greatly improved intestinal absorption and metabolic stability. Drug Metabolism and Disposition. 34, 1786-1792.

Zorloni, A., Penzhorn, B.L., Eloff, J.N., 2010. Extracts of *Calpurnia aurea* leaves from southern Ethiopia attract and immobilize or kill ticks. Veterinary Parasitology. 168, 160-164.

Chapter 4. CONCLUSION

The phytochemical analysis of *Sophora velutina* subsp. *zimbabweensis* yielded three novel quinolizidine alkaloids, 6,7-dihydroxylupanine and *N*-methylenehydroxycytisine isolated from fruits and pods, and velutinine from the stem bark along with the known quinolizidine alkaloids, *N*-methylcytisine, thermopsine, 7-hydroxylupanine, cytisine, and triterpenoids, lup-20(29)-ene-3β-ol and 12-oleanen-3-one and methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate.

It is important to note that the antibacterial activity studies against both *E. faecalis* and *P. aeruginosa* only compounds 12-oleanen-3-one and *N*-methylcytisine showed good activity against *E. faecalis* with MIC values of 10.9 and 20.8 μg mL^{-1}. The other compounds tested exhibited low to moderate antibacterial activity. This activity could be due to the *N*-methyl group in the quinolizidine alkaloid or the 3-keto group in the steroidal ketone. These compounds could make interesting subjects for structure-activity relationship studies with *E. faecalis*. These results could also validate the use of the plant as a mild antibacterial, however cytoxicity tests on the extract would need to be carried out before this becomes common practice as alkaloids are known to be cytotoxic. Derivatisation of the two active compounds to develop them into better antibiotics is a subject for future work.

Previous phytochemical studies reported in the literature on *Calpurnia aurea* was done on the roots and leaves of this plant. Hence we carried out a phytochemical analysis of wood and stem bark of this species. The results of the study resulted in the isolation of five isoflavanoids, a pterocarpan and a quinolizidine alkaloid. Isoflavones were only found in the seed of *C. aurea* previously and all the isoflavones isolated in this work as well as the pterocarpan were isolated for the first time from this source. These findings show the close

chemical relationship between the Podalyrieae and the Sophoreae in that three of the isoflavones were common to both tribes.

When the isoflavones were subjected to a variety of cancer cell lines they generally showed moderate activity against the renal, melanoma and breast cancer cell lines, with the 7-hydroxy-8-methoxy substitution on the chromone ring and 3'-hydroxy-4'-methoxy substitution on the phenyl ring as in compound 3',7-dihydroxy-4',8-dimethoxyisoflavone which had best activity.

SUPPORTING INFORMATION

The supporting information for this thesis is contained in two appendices. Appendix A contains the 1D and 2D NMR data for each of the compounds isolated from *Sophora velutina* as well as their IR, UV and MS data. Appendix B contains the same data for each of the compounds isolated from *Calpurnia aurea*.

Appendix A

NMR, UV, IR and MS data are presented for the following compounds isolated from *Sophora velutina* subsp. *zimbabweensis*

N-methylenehydroxycytisine **A1;** 7-hydroxylupanine **A2;** 6,7-dihydroxylupanine **A3;** 17-oxo-thermopsine **A4;** velutinine **A5;** N-methylcytisine **A6;** cytisine **A7**; methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate **A8;** lupeol **A9** (not UV and MS); 12-oleanen-3one **A10** (not MS)

Appendix B

NMR, UV, IR and MS data are presented for the following compounds isolated from *Calpurnia aurea*

7,3'dihydroxy-5'-methoxyisoflavone **B1**; 4',5,7-trihydroxyisoflavone **B2**; 7-hydroxy-4',8-dimethoxyisoflavone **B3**; 7-acetoxy-4',8-dimethoxyisoflavone **B4**; 3',7-dihydroxy-4',8-dimethoxyisoflavone **B5**; 3-acetoxy-9-methoxypterocarpan **B6**; calpurnine **B7**

Appendix A

NMR, UV, IR and MS data are presented for the following compounds isolated from *Sophora velutina* subsp. *zimbabweensis*

N-methylenehydroxycytisine **A1**

7-hydroxylupanine **A2**

6,7-dihydroxylupanine **A3**

17-oxo-thermopsine **A4**

velutinine **A5**

N-methylcytisine **A6**

cytisine **A7**

methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate **A8**

lupeol **A9** (not UV and MS)

12-oleanen-3one **A10** (not MS)

Appendix B

NMR, UV, IR and MS data for compounds isolated from *Calpurnia aurea*

7,3'dihydroxy-5'-methoxyisoflavone **B1**

4',5,7-trihydroxyisoflavone **B2**

7-hydroxy-4',8-dimethoxyisoflavone **B3**

7-acetoxy-4',8-dimethoxyisoflavone **B4**

3',7-dihydroxy-4',8-dimethoxyisoflavone **B5**

3-acetoxy-9-methoxypterocarpan **B6**

calpurnine **B7**

SUPPORTING INFORMATION

The supporting information for this thesis is contained in two appendices. Appendix A contains the 1D and 2D NMR data for each of the compounds isolated from *Sophora velutina* as well as their IR, UV and MS data. Appendix B contains the same data for each of the compounds isolated from *Calpurnia aurea*.

Appendix A

NMR, UV, IR and MS data are presented for the following compounds isolated from *Sophora velutina* subsp. *zimbabweensis*

N-methylenehydroxycytisine **A1;** 7-hydroxylupanine **A2;** 6,7-dihydroxylupanine **A3;** 17-oxo-thermopsine **A4;** velutinine **A5;** N-methylcytisine **A6;** cytisine **A7**; methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate **A8;** lupeol **A9** (not UV and MS); 12-oleanen-3one **A10** (not MS)

Appendix B

NMR, UV, IR and MS data are presented for the following compounds isolated from *Calpurnia aurea*

7,3'dihydroxy-5'-methoxyisoflavone **B1**; 4',5,7-trihydroxyisoflavone **B2**; 7-hydroxy-4',8-dimethoxyisoflavone **B3**; 7-acetoxy-4',8-dimethoxyisoflavone **B4**; 3',7-dihydroxy-4',8-dimethoxyisoflavone **B5**; 3-acetoxy-9-methoxypterocarpan **B6**; calpurnine **B7**

Appendix A

NMR, UV, IR and MS data are presented for the following compounds isolated from *Sophora velutina* subsp. *zimbabweensis*

N-methylenehydroxycytisine **A1**

7-hydroxylupanine **A2**

6,7-dihydroxylupanine **A3**

17-oxo-thermopsine **A4**

velutinine **A5**

N-methylcytisine **A6**

cytisine **A7**

methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate **A8**

lupeol **A9** (not UV and MS)

12-oleanen-3one **A10** (not MS)

Appendix B

NMR, UV, IR and MS data for compounds isolated from *Calpurnia aurea*

7,3'dihydroxy-5'-methoxyisoflavone **B1**

4',5,7-trihydroxyisoflavone **B2**

7-hydroxy-4',8-dimethoxyisoflavone **B3**

7-acetoxy-4',8-dimethoxyisoflavone **B4**

3',7-dihydroxy-4',8-dimethoxyisoflavone **B5**

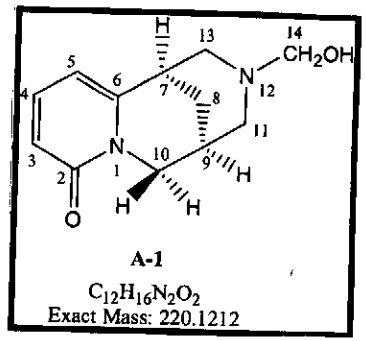
3-acetoxy-9-methoxypterocarpan **B6**

calpurnine **B7**

hsvx1.svsmb xxx1/5/9/06 in cdcl3
with presat_h2o
satpwr=-14
probe=5mmASW

Pulse Sequence: presat_da

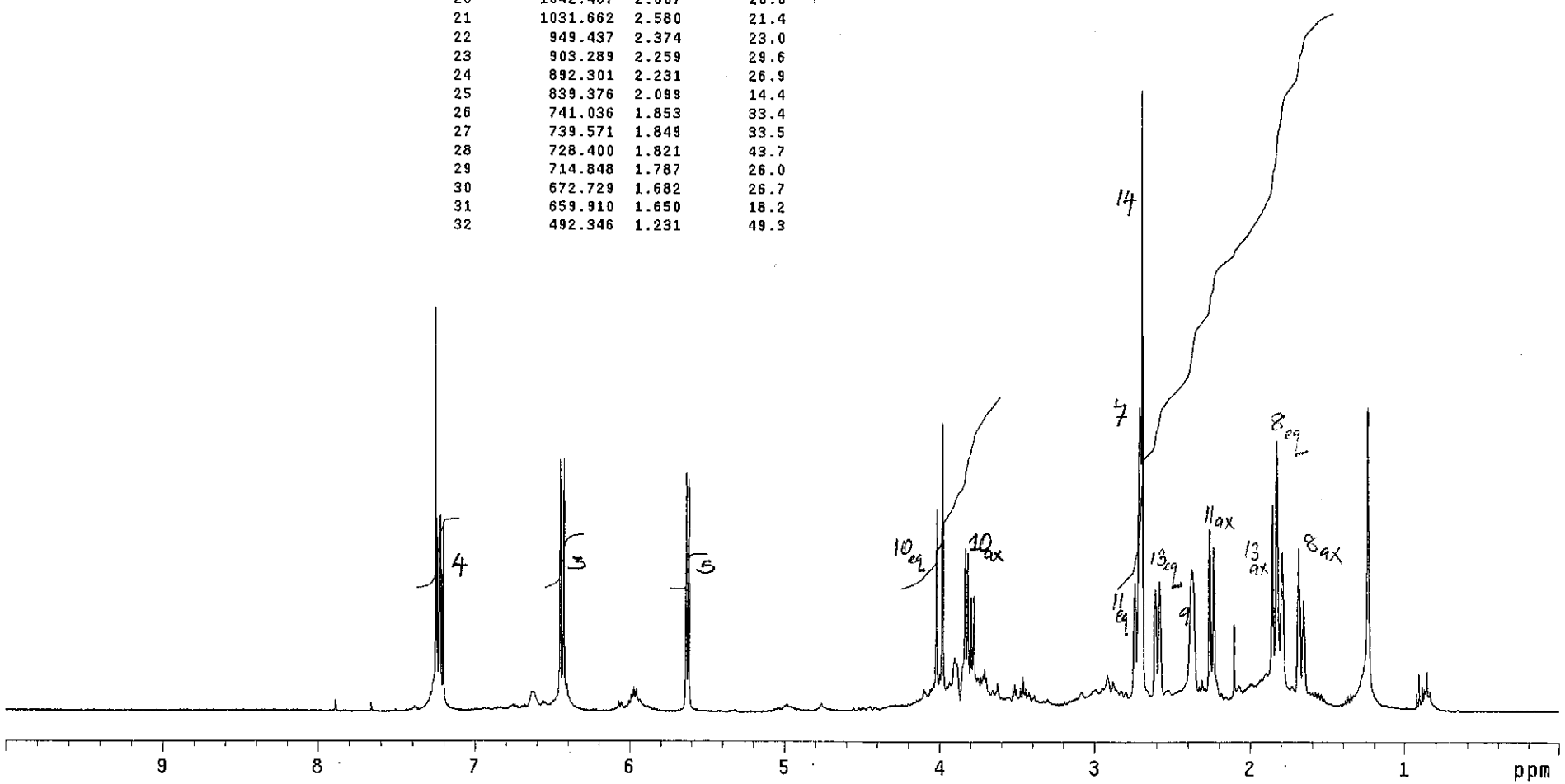
INDEX	FREQUENCY	PPM	HEIGHT
1	2899.765	7.251	64.8
2	2898.849	7.248	58.6
3	2895.736	7.240	31.2
4	2888.777	7.223	31.6
5	2886.763	7.218	31.8
6	2879.804	7.201	30.0
7	2578.190	6.446	40.6
8	2569.216	6.424	40.8
9	2254.051	5.636	38.4
10	2247.275	5.619	37.5
11	1607.421	4.019	32.7
12	1592.038	3.981	46.6
13	1533.070	3.833	26.5
14	1526.478	3.817	25.9
15	1517.871	3.795	18.7
16	1511.095	3.778	18.8
17	1095.025	2.738	21.1
18	1082.755	2.707	49.3
19	1076.346	2.691	100.0
20	1042.467	2.607	20.0
21	1031.662	2.580	21.4
22	949.437	2.374	23.0
23	903.289	2.259	29.6
24	892.301	2.231	26.9
25	839.376	2.099	14.4
26	741.036	1.853	33.4
27	739.571	1.849	33.5
28	728.400	1.821	43.7
29	714.848	1.787	26.0
30	672.729	1.682	26.7
31	659.910	1.650	18.2
32	492.346	1.231	49.3



A-1

$C_{12}H_{16}N_2O_2$

Exact Mass: 220.1212

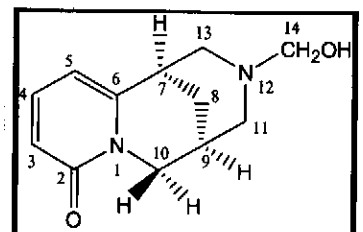


^{1}H NMR spectrum of *N*-methylenehydroxycytisine A1

csvx1.svsmb xxx1/5/9/06 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

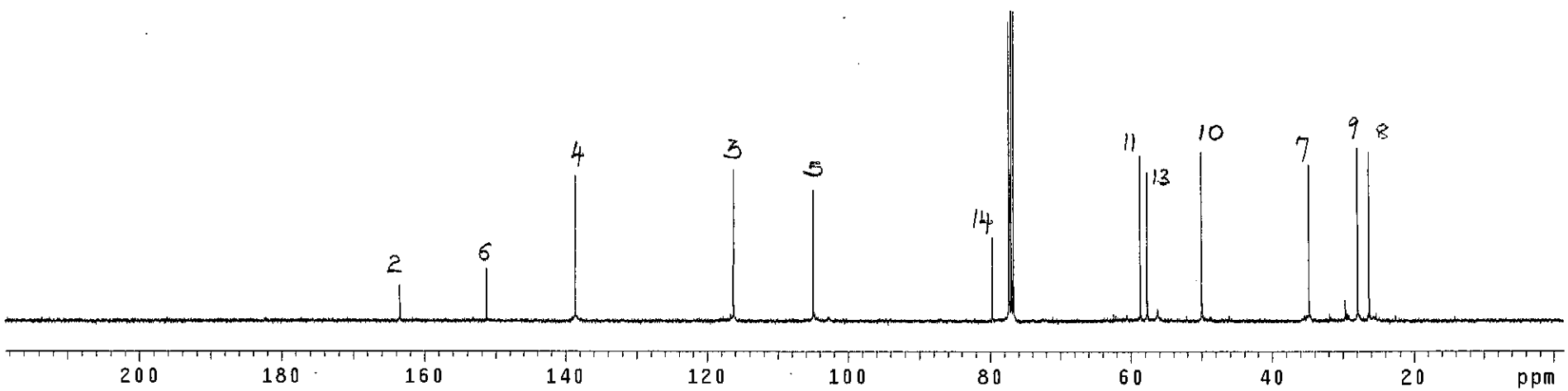
INDEX	FREQUENCY	PPM	HEIGHT
1	16440.483	163.482	6.0
2	15216.691	151.313	8.7
3	13937.202	138.590	23.5
4	11690.277	116.246	24.5
5	10554.988	104.957	21.5
6	8014.322	79.693	13.8
7	7775.515	77.319	48.2
8	7743.471	77.000	50.0
9	7711.426	76.681	49.9
10	5900.153	58.670	27.0
11	5800.968	57.684	24.0
12	5023.509	49.953	27.6
13	3493.768	34.742	25.4
14	2800.998	27.853	28.2
15	2637.724	26.229	27.6



A-1

$C_{12}H_{16}N_2O_2$

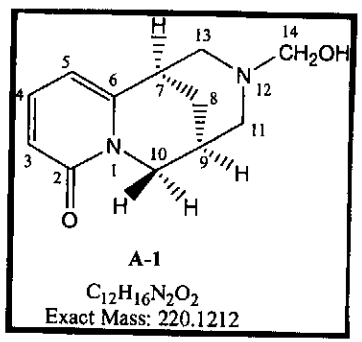
Exact Mass: 220.1212



^{13}C NMR spectrum of *N*-methylenehydroxycytisine A1

dsvx1.svsmb xxx1/5/9/06 in cdcl3
probe=5mmASW

Pulse Sequence: dept



A-1

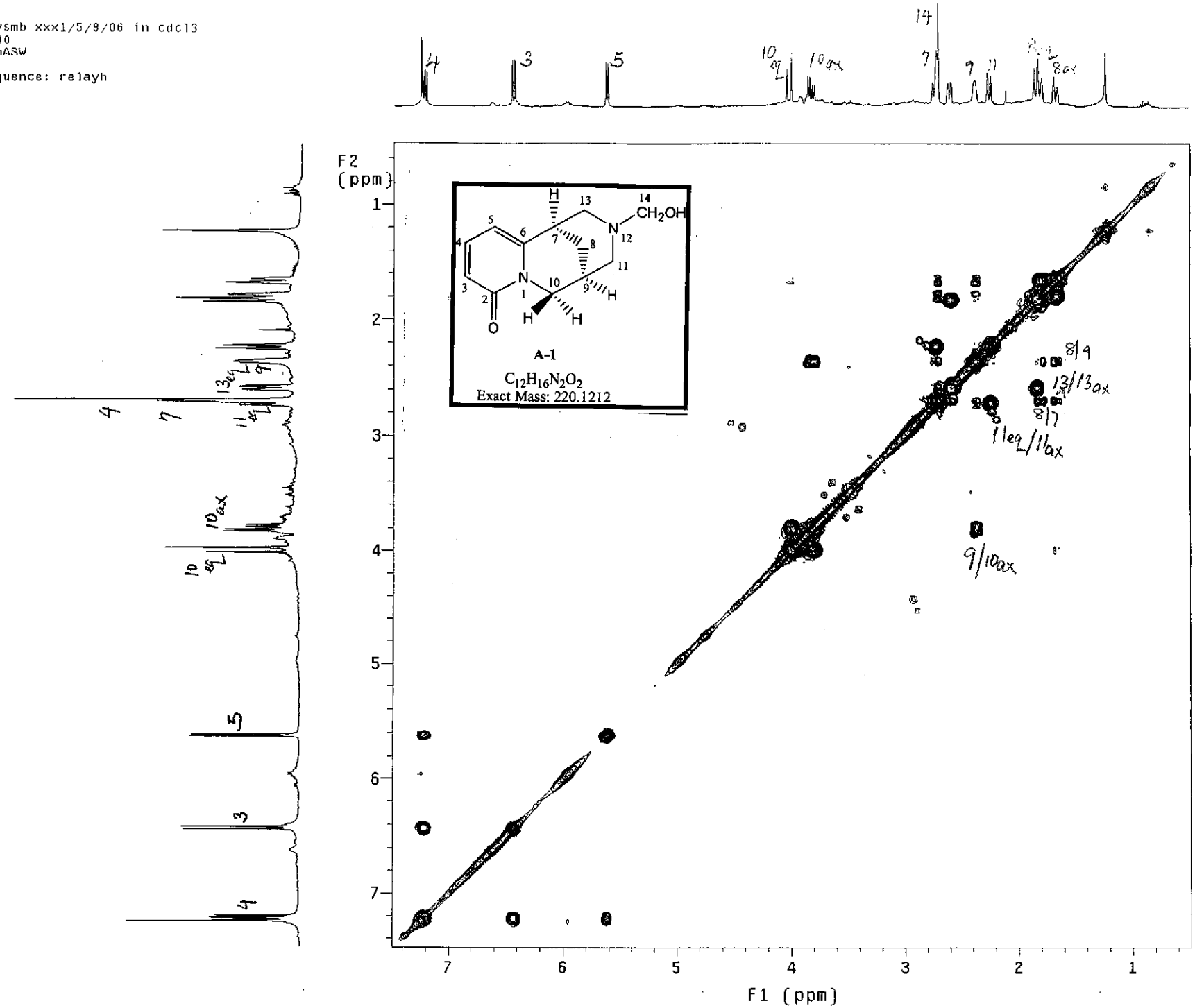
$C_{12}H_{16}N_2O_2$

Exact Mass: 220.1212

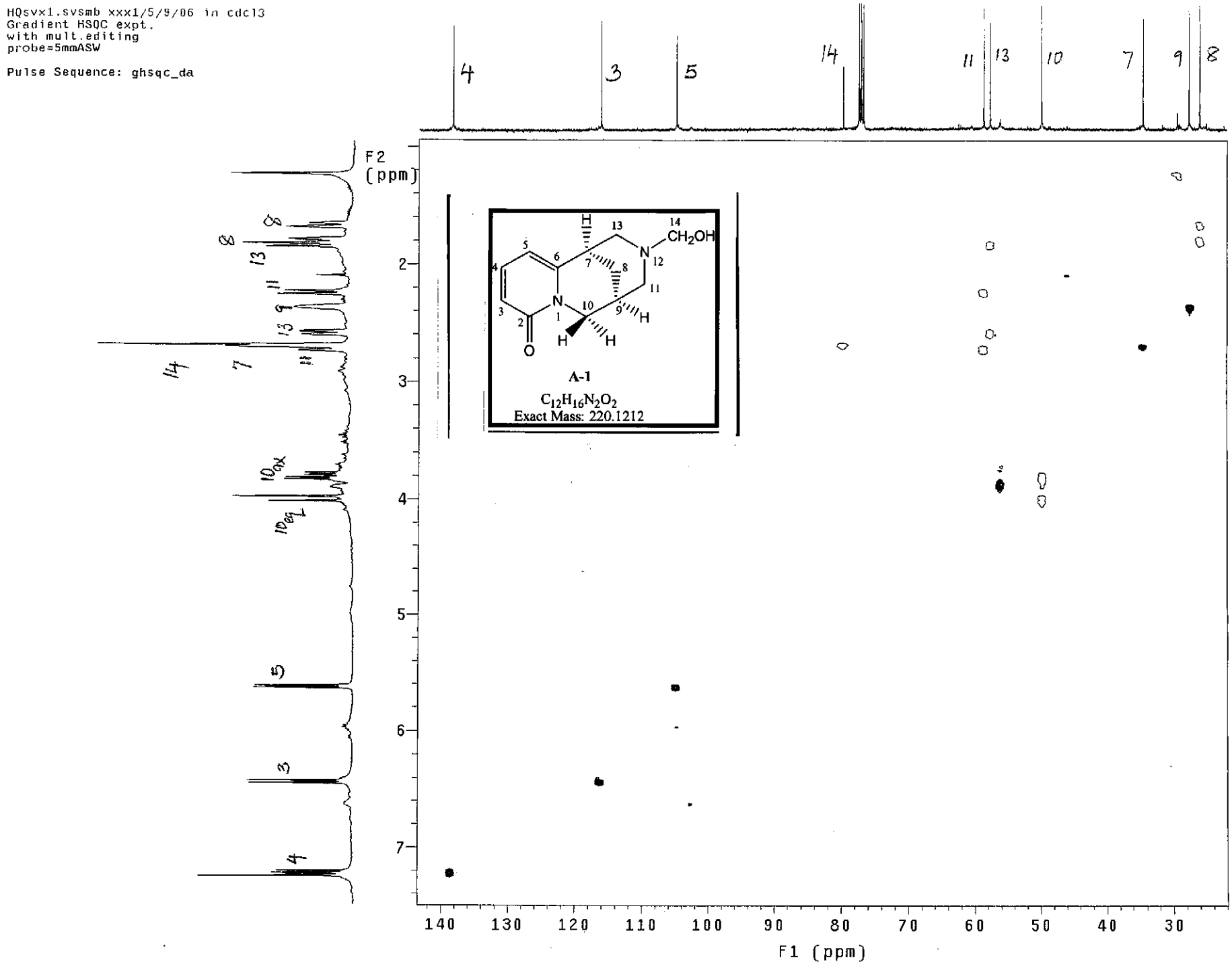
DEPT spectrum of *N*-methylenehydroxycytisine A1

cysvx1.svsmb xxx1/5/9/06 in cdcl3
1H Cosy-90
probe=5mmASW

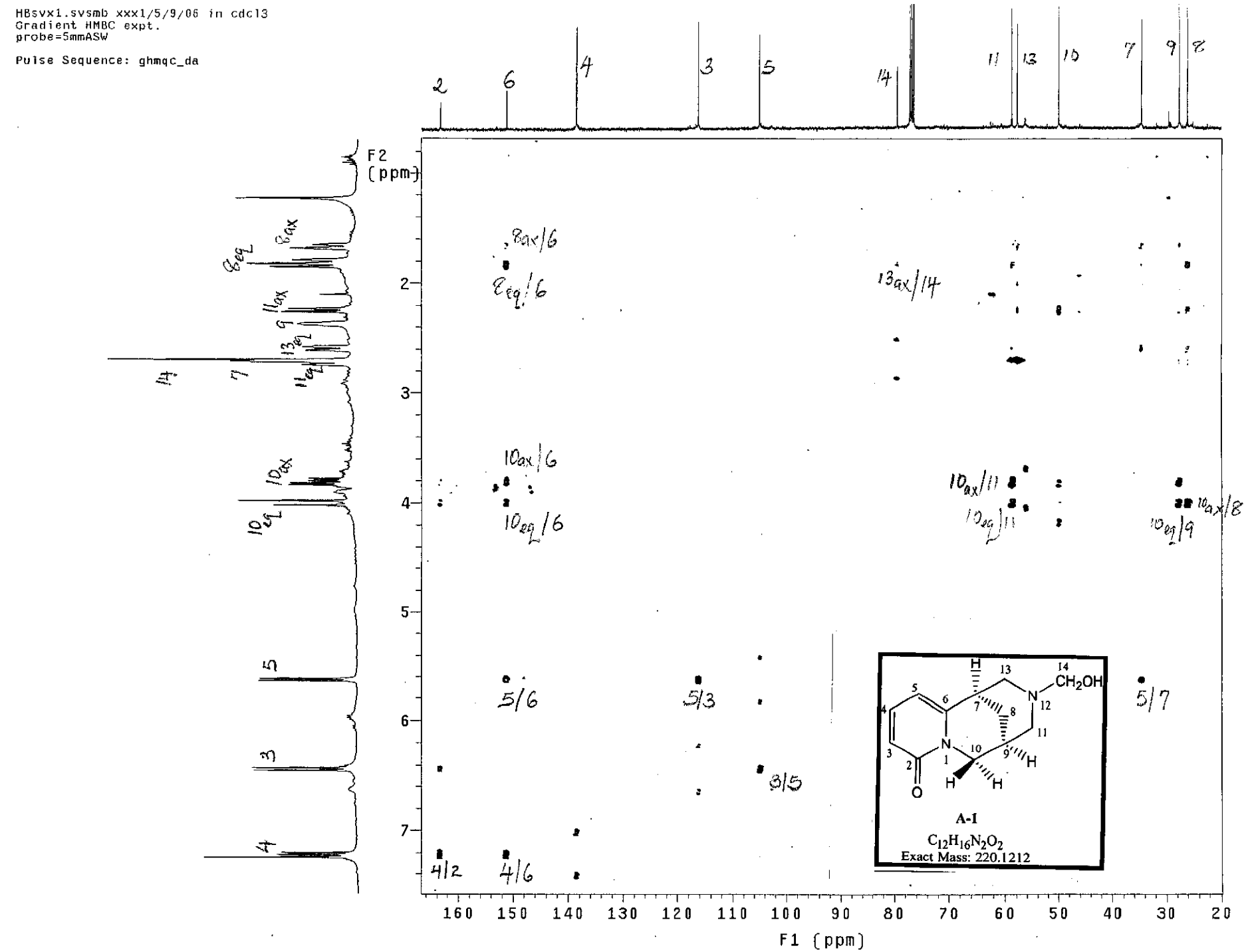
Pulse Sequence: relayh



COSY spectrum of *N*-methylenehydroxycytisine A1



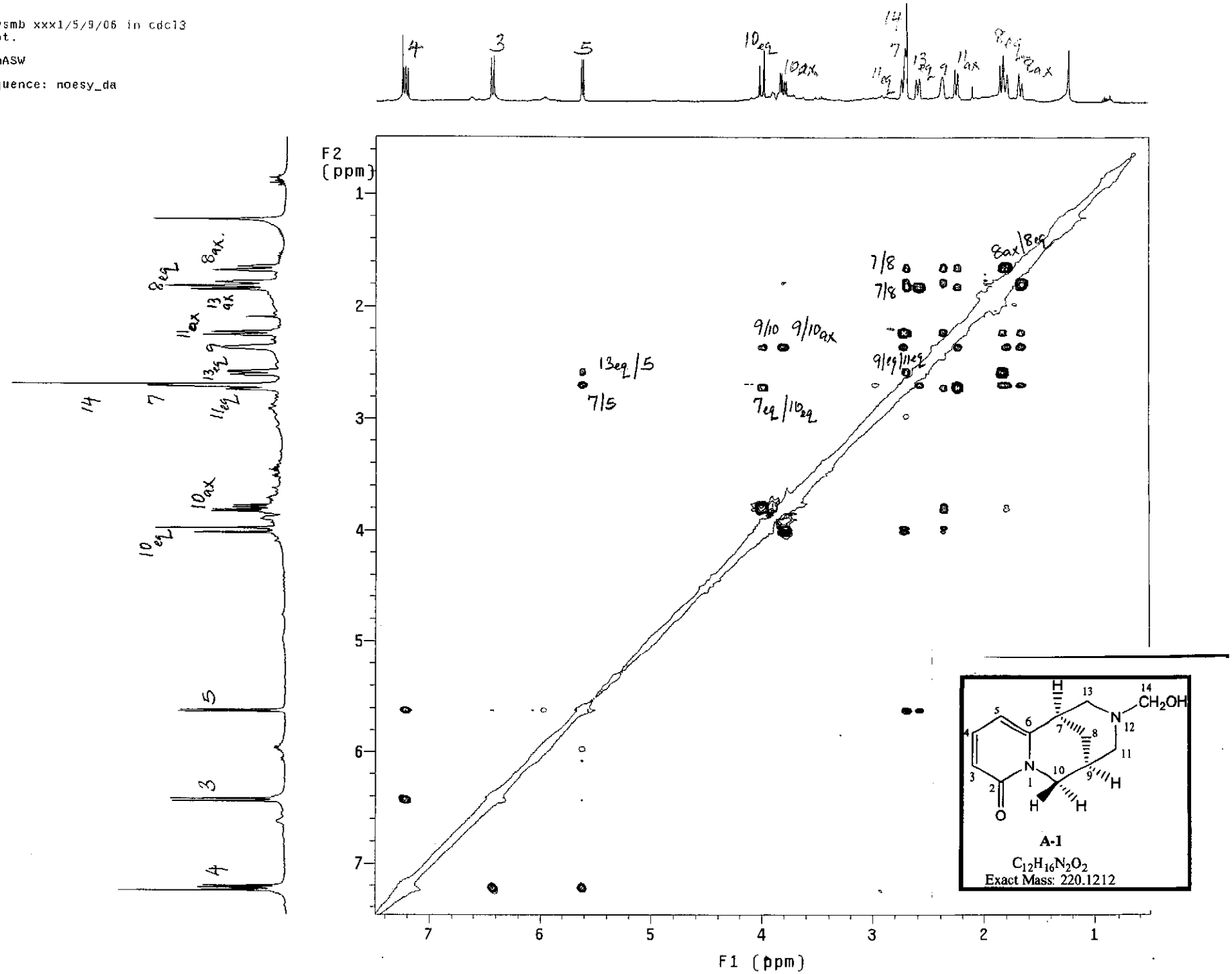
HSQC spectrum of *N*-methylenehydroxycytisine A1



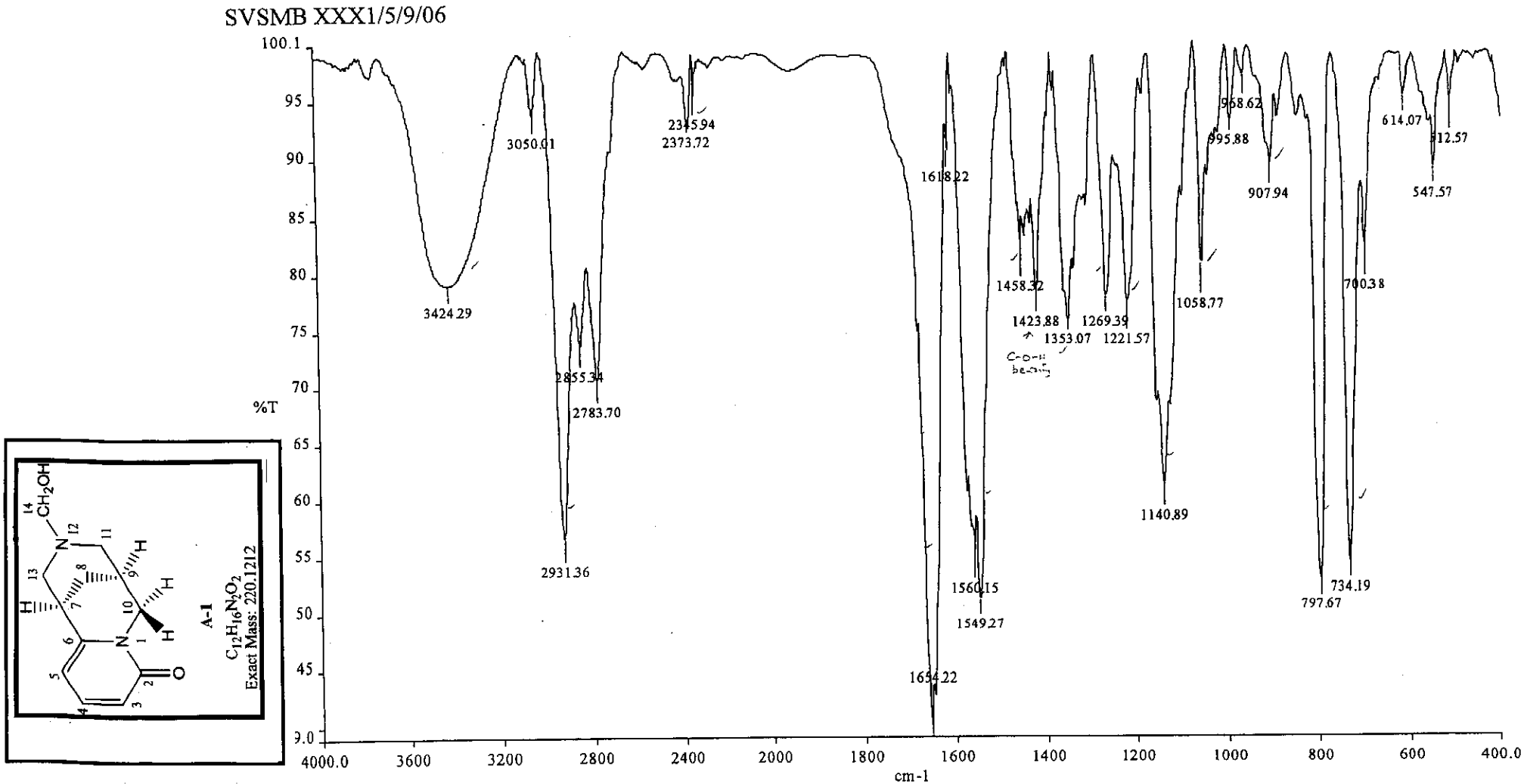
HMBC spectrum of *N*-methylenehydroxycytisine A1

NOsvx1.svsmb xxx1/5/9/06 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW

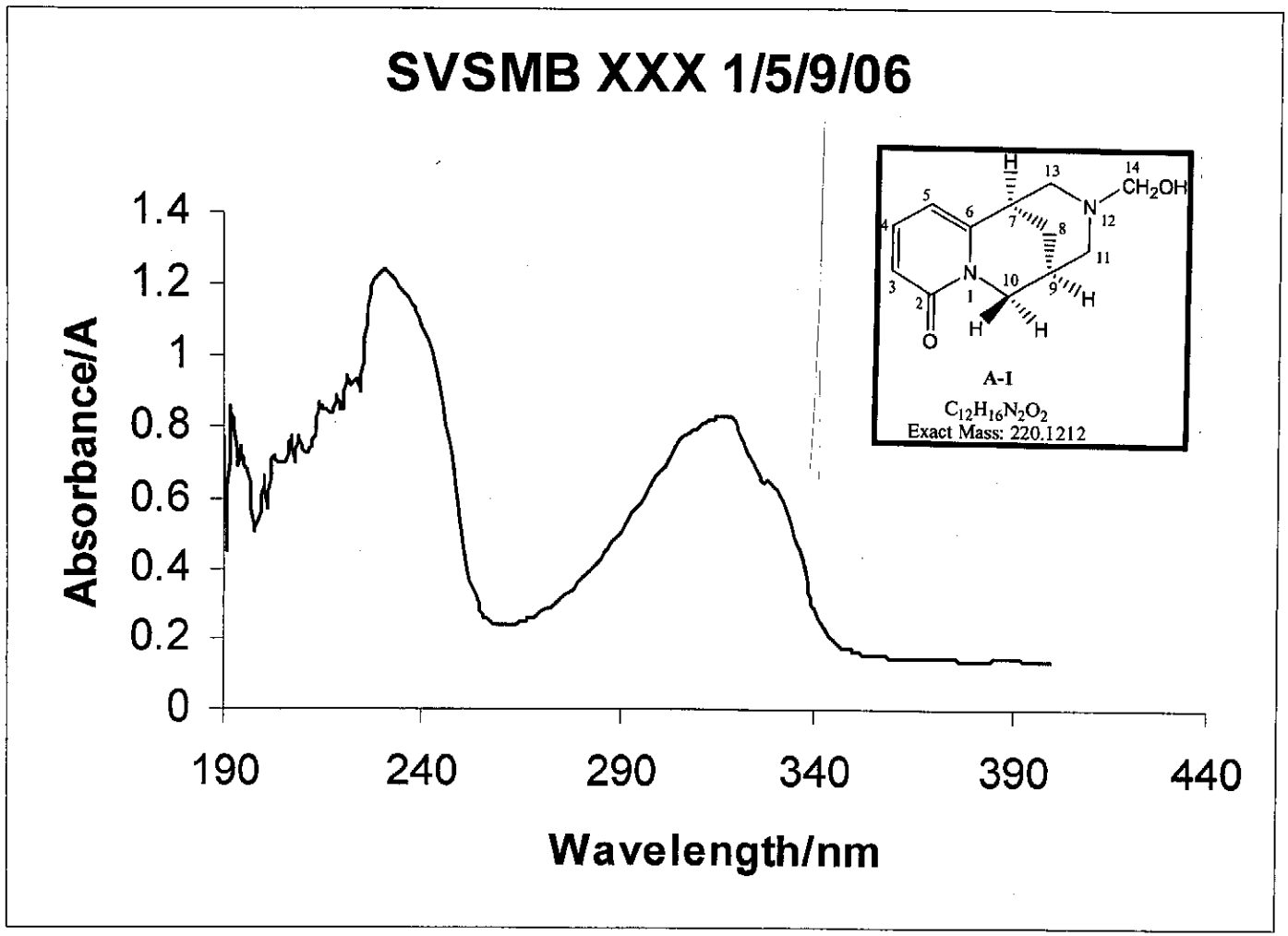
Pulse Sequence: noesy_da



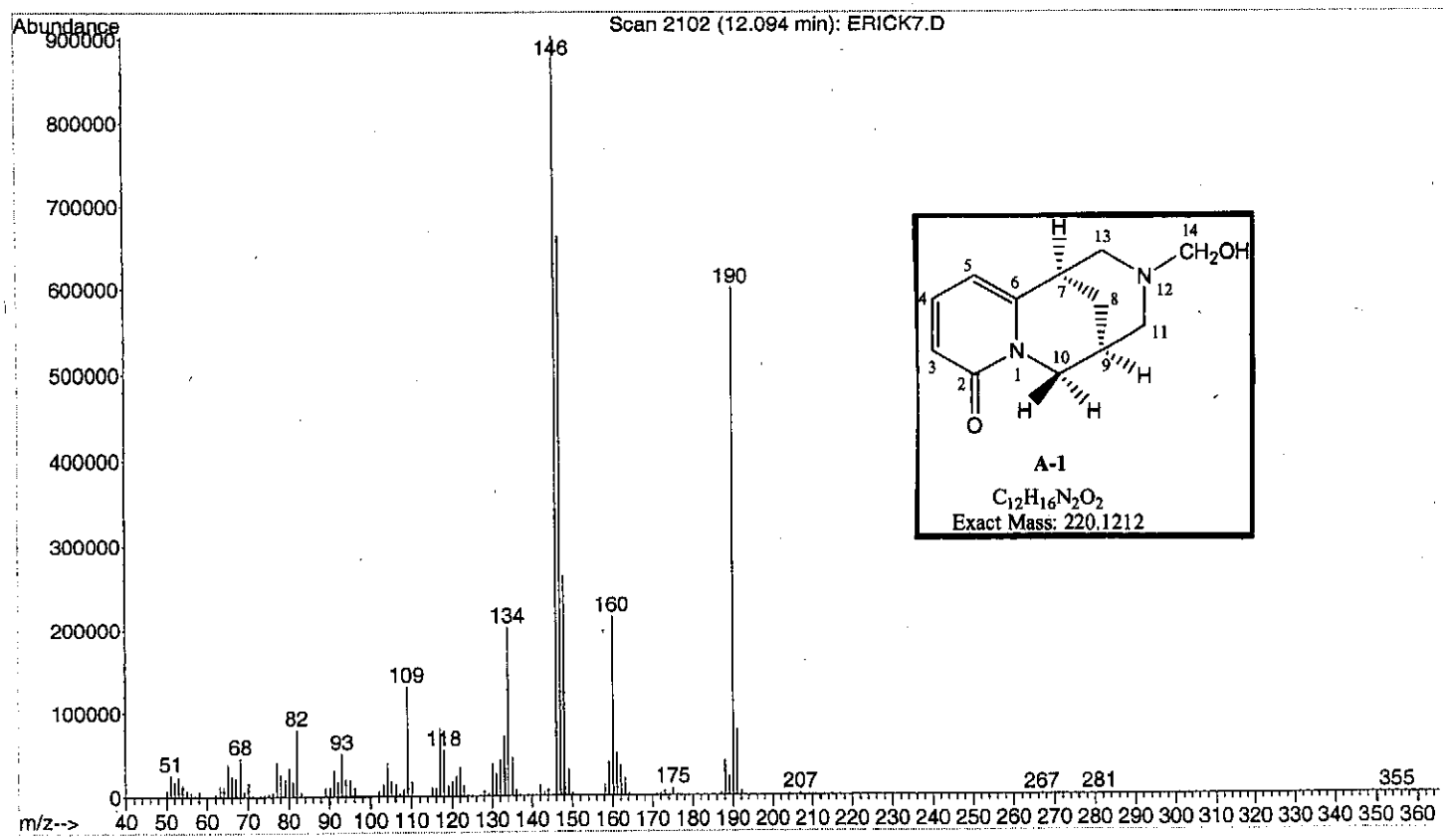
NOESY spectrum of *N*-methylenehydroxycytisine A1



IR spectrum of *N*-methylenehydroxycytisine A1



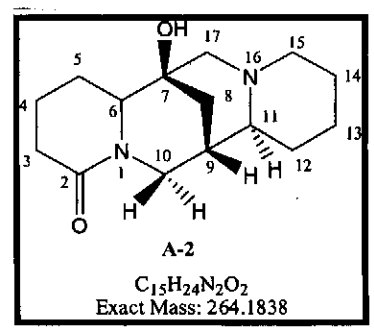
UV spectrum of *N*-methylenehydroxycytisine A1



Mass spectrum of *N*- methylenehydroxycytisine A1

hsv337.svma 32-33.7 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul



A-2

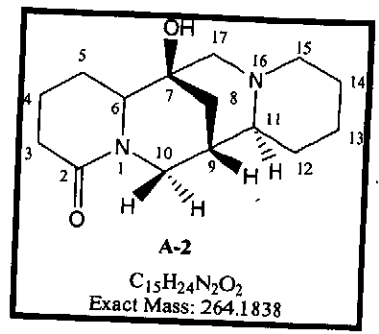
$C_{15}H_{24}N_2O_2$
Exact Mass: 264.1838

^{1}H NMR spectrum of -7-hydroxylupanine A2

hsv337.svma 32-33.7 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2895.553	7.240	23.8	40	649.838	1.625	9.7	79	369.833	0.925	11.0
2	1503.587	3.760	12.7	41	646.358	1.616	9.6				
3	1498.459	3.747	20.1	42	643.611	1.609	9.9				
4	1494.064	3.736	19.0	43	641.597	1.604	9.8				
5	1490.584	3.727	41.7	44	637.934	1.595	11.5				
6	1484.175	3.711	48.8	45	632.074	1.580	27.4				
7	1479.414	3.699	7.1	46	630.060	1.575	25.7				
8	1474.652	3.687	30.0	47	621.636	1.554	33.4				
9	1471.356	3.679	9.4	48	619.804	1.550	31.9				
10	1461.833	3.655	10.5	49	612.113	1.531	12.5				
11	1182.195	2.956	12.5	50	604.238	1.511	7.1				
12	1178.631	2.950	12.4	51	595.265	1.488	7.5				
13	1173.221	2.934	12.5	52	591.602	1.479	14.0				
14	1171.390	2.929	13.2	53	587.757	1.470	9.1				
15	1169.009	2.923	12.5	54	582.446	1.456	6.3				
16	1098.138	2.746	14.2	55	578.783	1.447	12.1				
17	1086.784	2.717	15.5	56	574.754	1.437	9.7				
18	1050.891	2.628	87.7	57	568.528	1.422	16.1				
19	1044.481	2.612	150.0	58	565.415	1.414	13.2				
20	1037.889	2.595	94.1	59	556.442	1.391	13.2				
21	994.670	2.487	5.8	60	553.511	1.384	11.7				
22	827.107	2.068	11.1	61	539.227	1.348	5.4				
23	824.177	2.061	16.2	62	529.155	1.323	14.2				
24	815.203	2.038	27.3	63	525.676	1.314	9.1				
25	812.456	2.031	27.5	64	518.717	1.297	9.7				
26	803.483	2.009	34.7	65	515.604	1.289	13.0				
27	800.553	2.002	22.4	66	512.490	1.281	7.0				
28	792.862	1.982	8.9	67	505.348	1.264	6.4				
29	789.565	1.974	14.0	68	502.418	1.256	6.1				
30	787.001	1.968	13.9	69	487.402	1.219	8.6				
31	770.703	1.927	13.5	70	483.739	1.210	9.8				
32	764.110	1.911	36.7	71	476.048	1.190	12.0				
33	759.898	1.900	38.2	72	472.751	1.182	13.2				
34	757.701	1.895	50.8	73	464.694	1.162	9.0				
35	750.009	1.875	54.3	74	460.848	1.152	9.9				
36	744.699	1.862	10.7	75	394.921	0.987	6.1				
37	739.205	1.848	22.0	76	386.314	0.966	13.2				
38	659.177	1.648	6.9	77	382.286	0.956	12.4				
39	654.965	1.638	8.8	78	374.045	0.935	11.4				



A-2

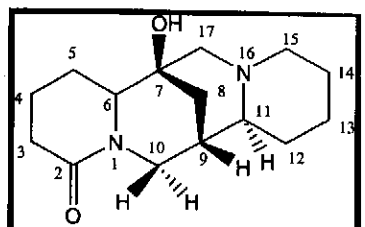
$C_{15}H_{24}N_2O_2$

Exact Mass: 264.1838

csv337.svma 32-33.7 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

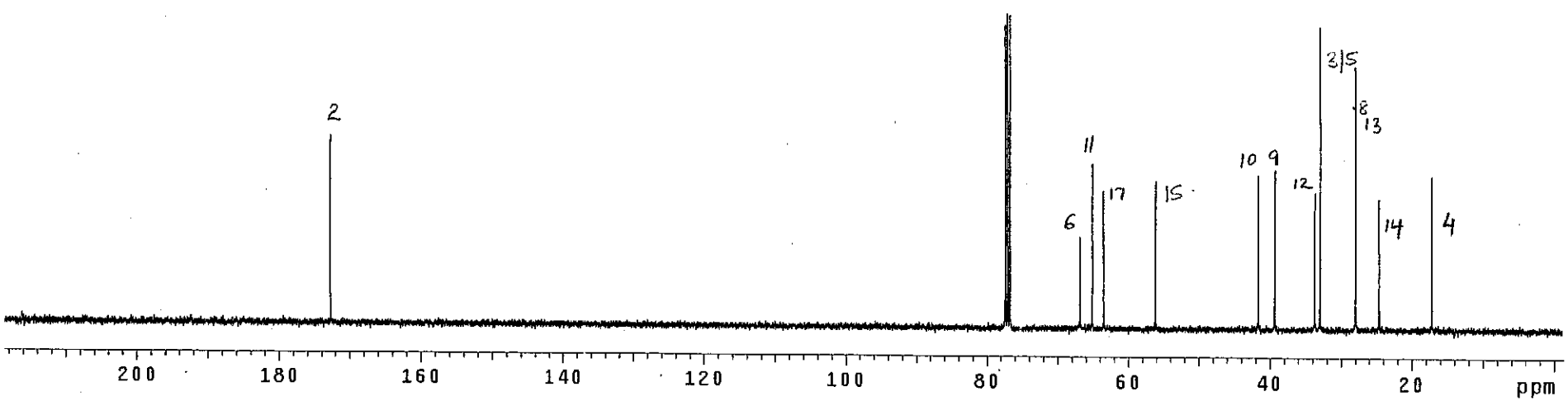
INDEX	FREQUENCY	PPM	HEIGHT
1	17369.009	172.715	29.8
2	7775.515	77.319	48.1
3	7743.471	77.000	50.0
4	7711.427	76.681	49.8
5	6715.760	66.781	14.7
6	6540.279	65.036	26.1
7	6378.531	63.427	22.0
8	5640.746	56.091	23.6
9	4178.909	41.554	24.5
10	3944.679	39.225	25.3
11	3382.376	33.634	21.9
12	3304.554	32.860	48.1
13	2803.287	27.875	41.8
14	2470.635	24.568	20.9
15	1718.354	17.087	24.6



A-2

$C_{15}H_{24}N_2O_2$
Exact Mass: 264.1838

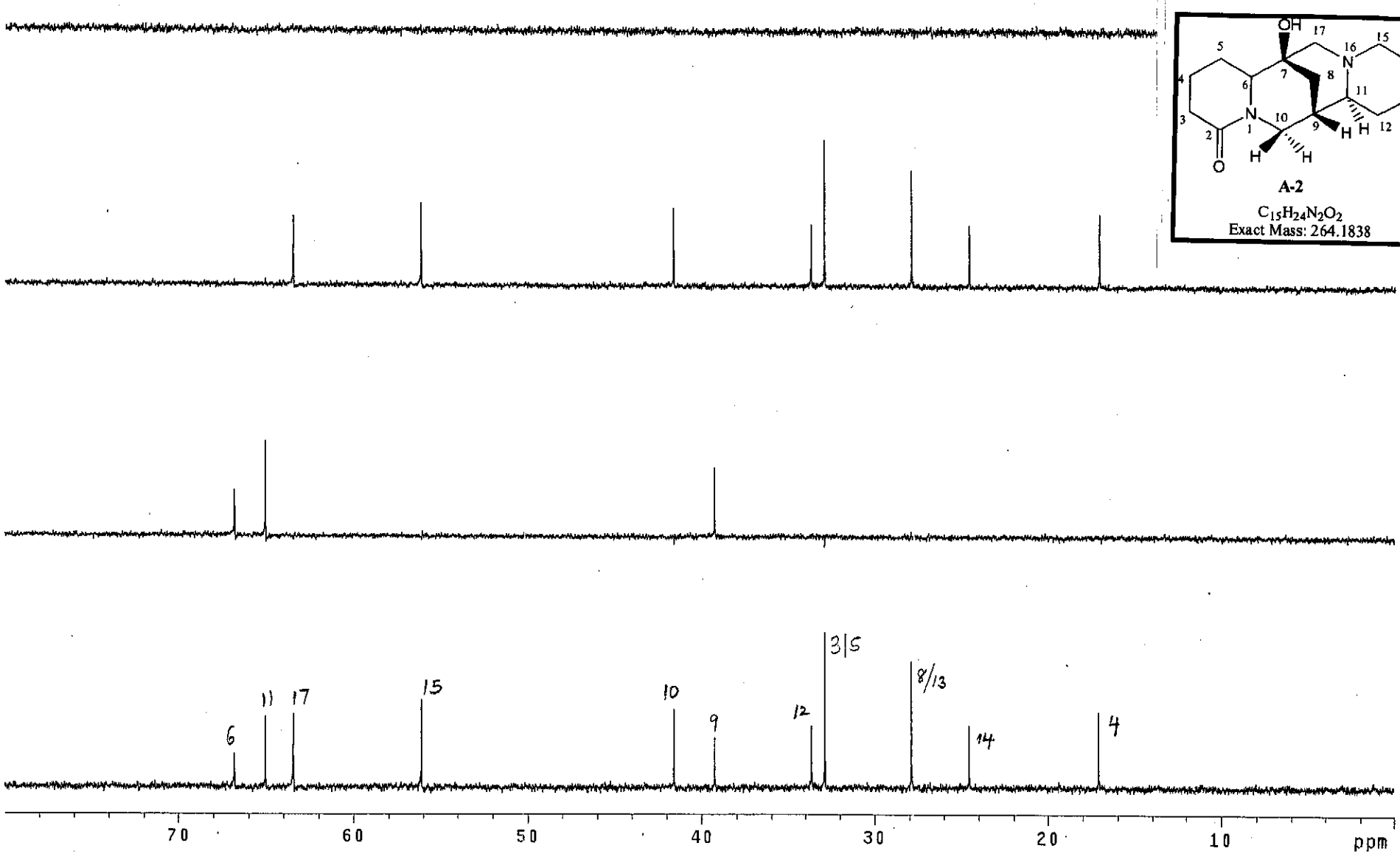
* C-7 could not be detected. assumed to be overlapping with the solvent peak.



^{13}C NMR spectrum of -7-hydroxylupanine A2

dsv337.svma 32-33.7 in cdcl3
probe=5mmASW

Pulse Sequence: dept



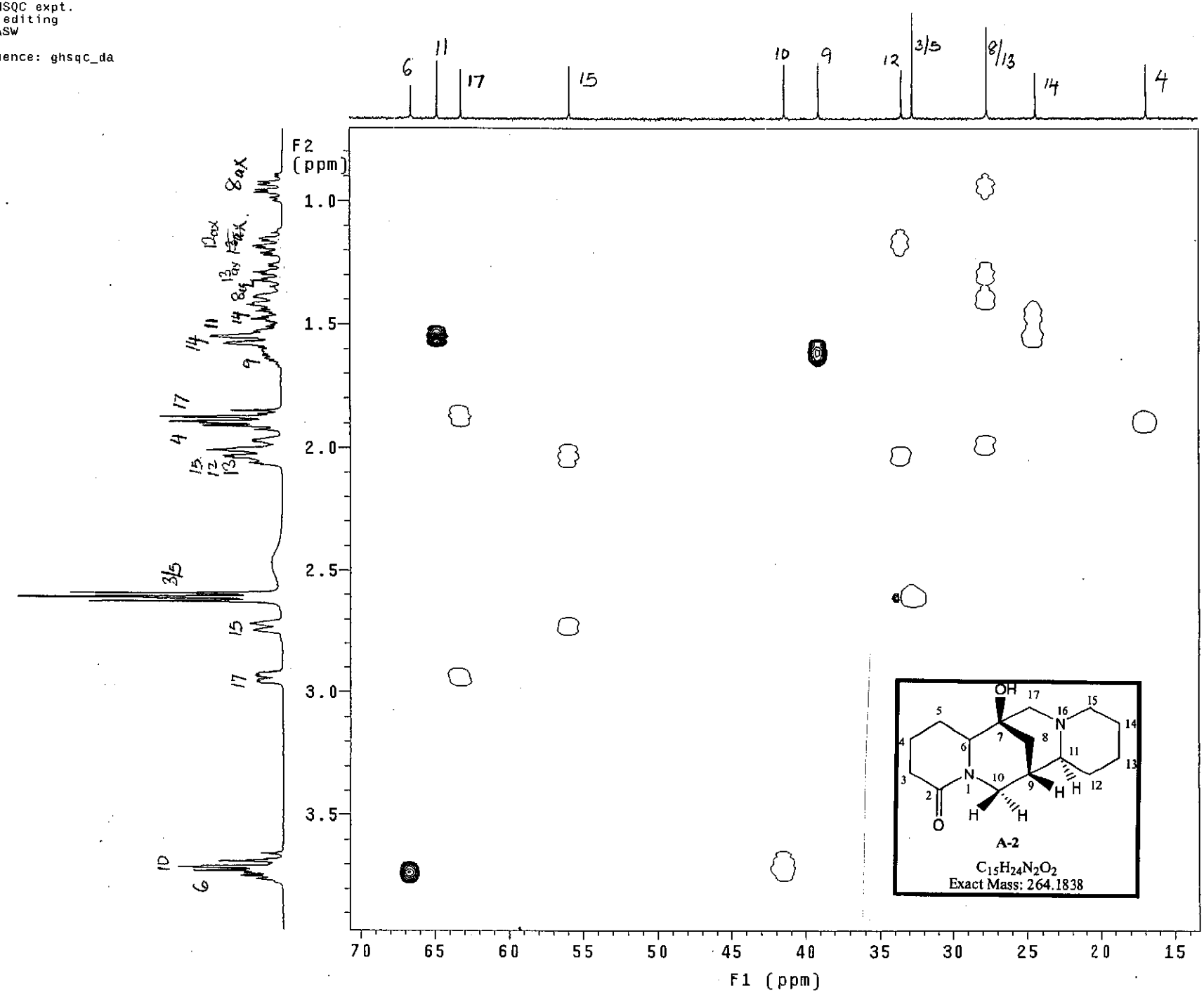
A-2

$C_{15}H_{24}N_2O_2$
Exact Mass: 264.1838

DEPT spectrum of -7-hydroxylupanine A2

HQsv337.svma 32-33.7 in cdcl3
Gradient HSQC expt.
with mult.editing
probe=5mmASW

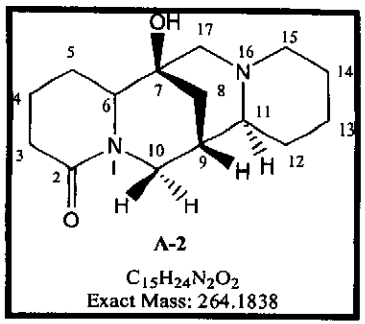
Pulse Sequence: ghsqc_da



HSQC spectrum of -7-hydroxylupanine A2

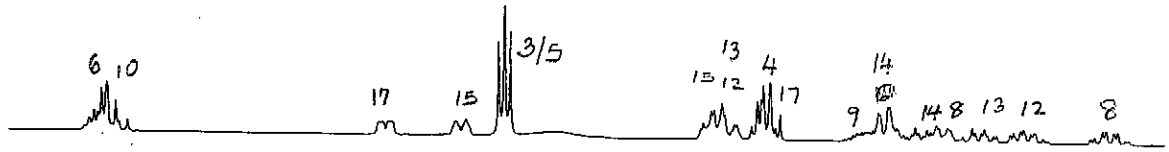
cysv337.svma 32-33.7 in cdcl3
1H Cosy-90
probe=5mmASW

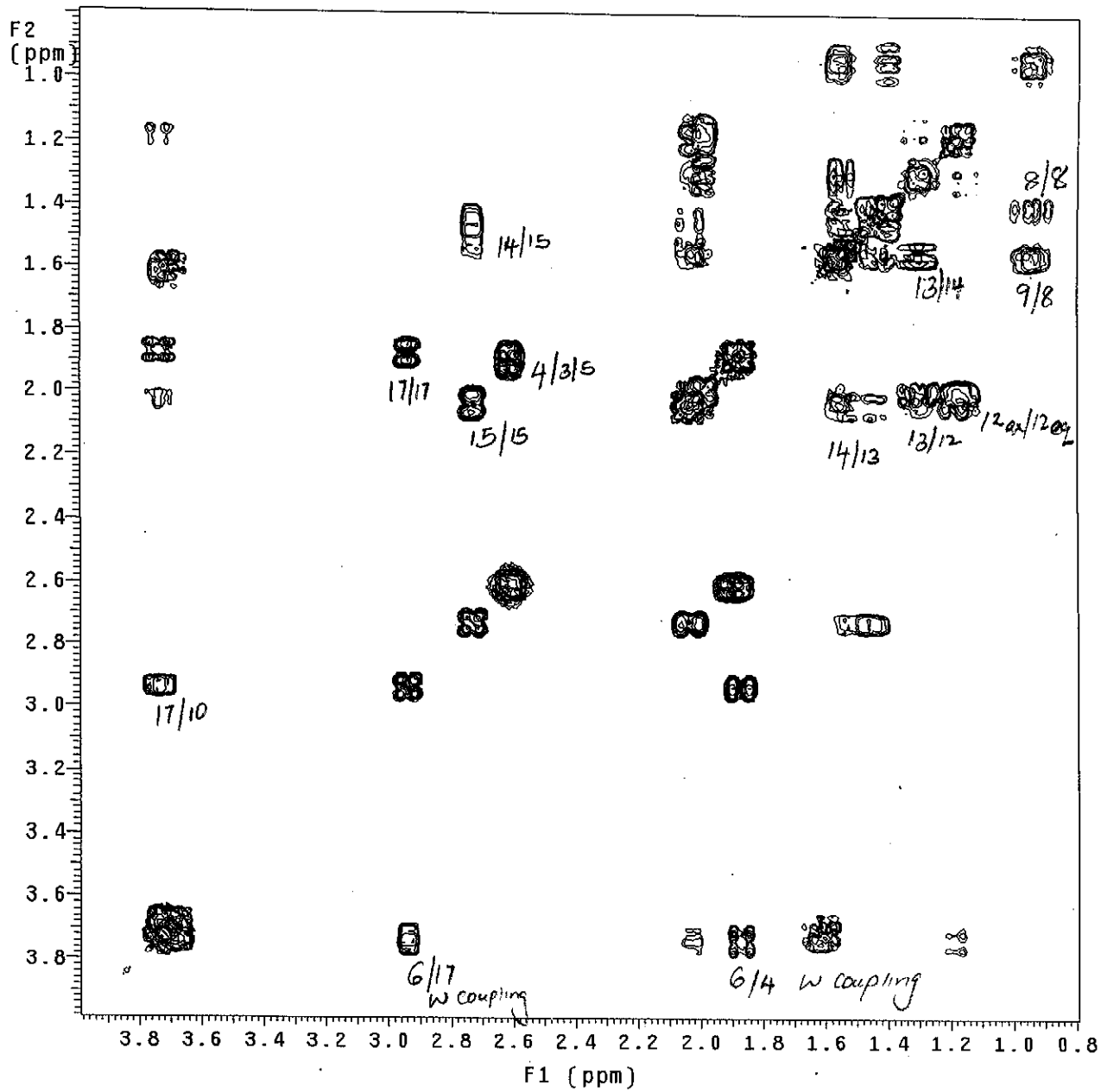
Pulse Sequence: relayh



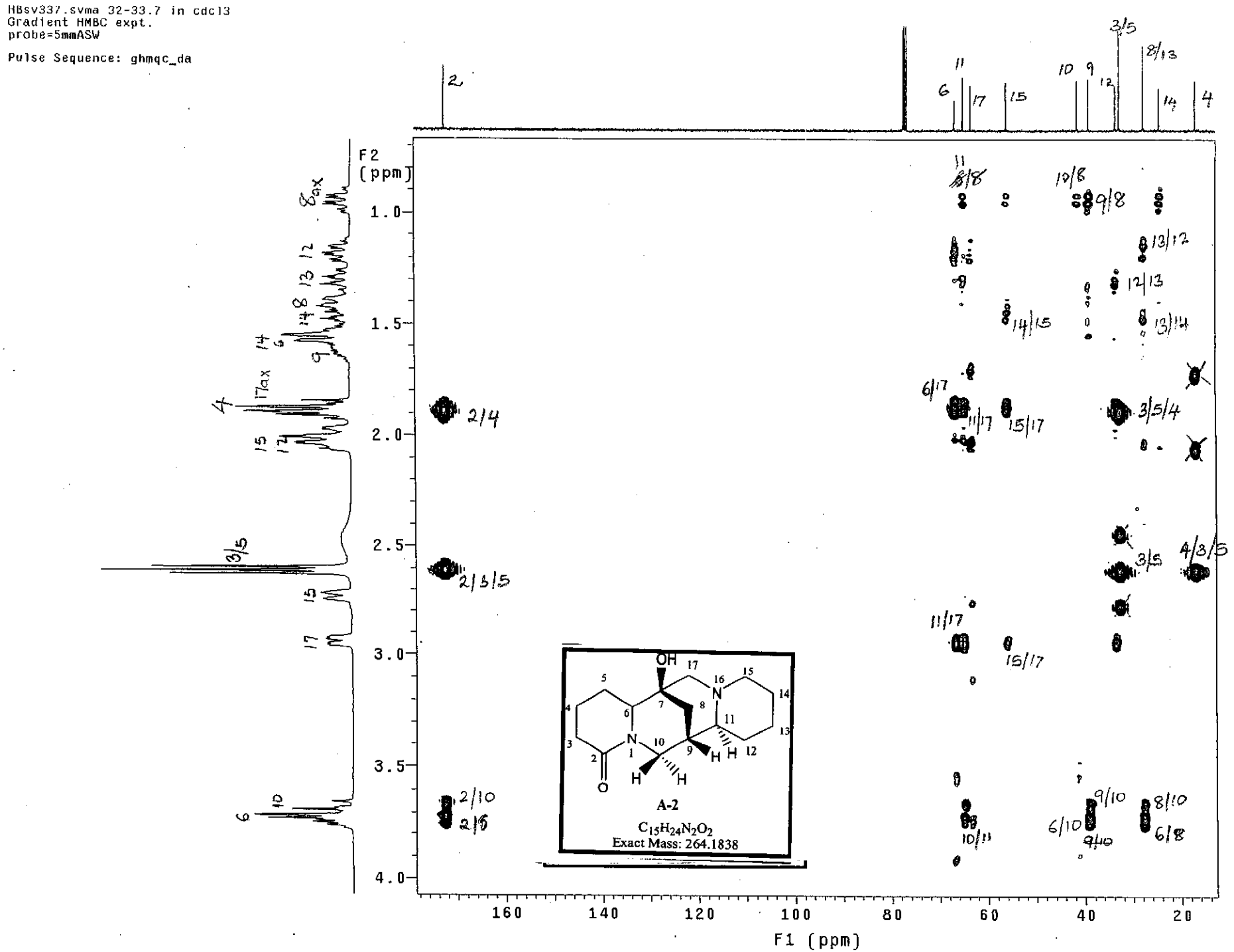
A-2

$C_{15}H_{24}N_2O_2$
Exact Mass: 264.1838





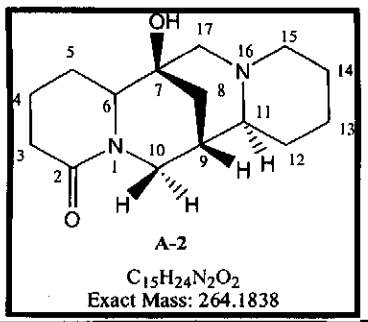
COSY spectrum of -7-hydroxylupanine A2



HMBC spectrum of -7-hydroxylupanine A2

NOsv337.svma 32-33.7 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW

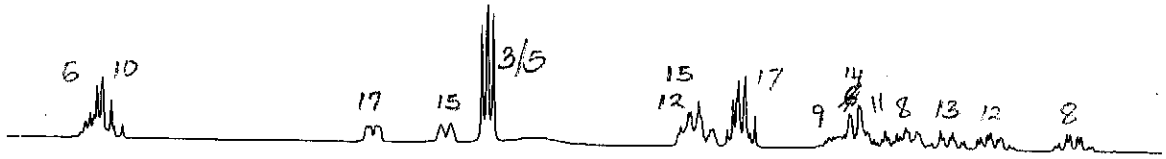
Pulse Sequence: noesy_da

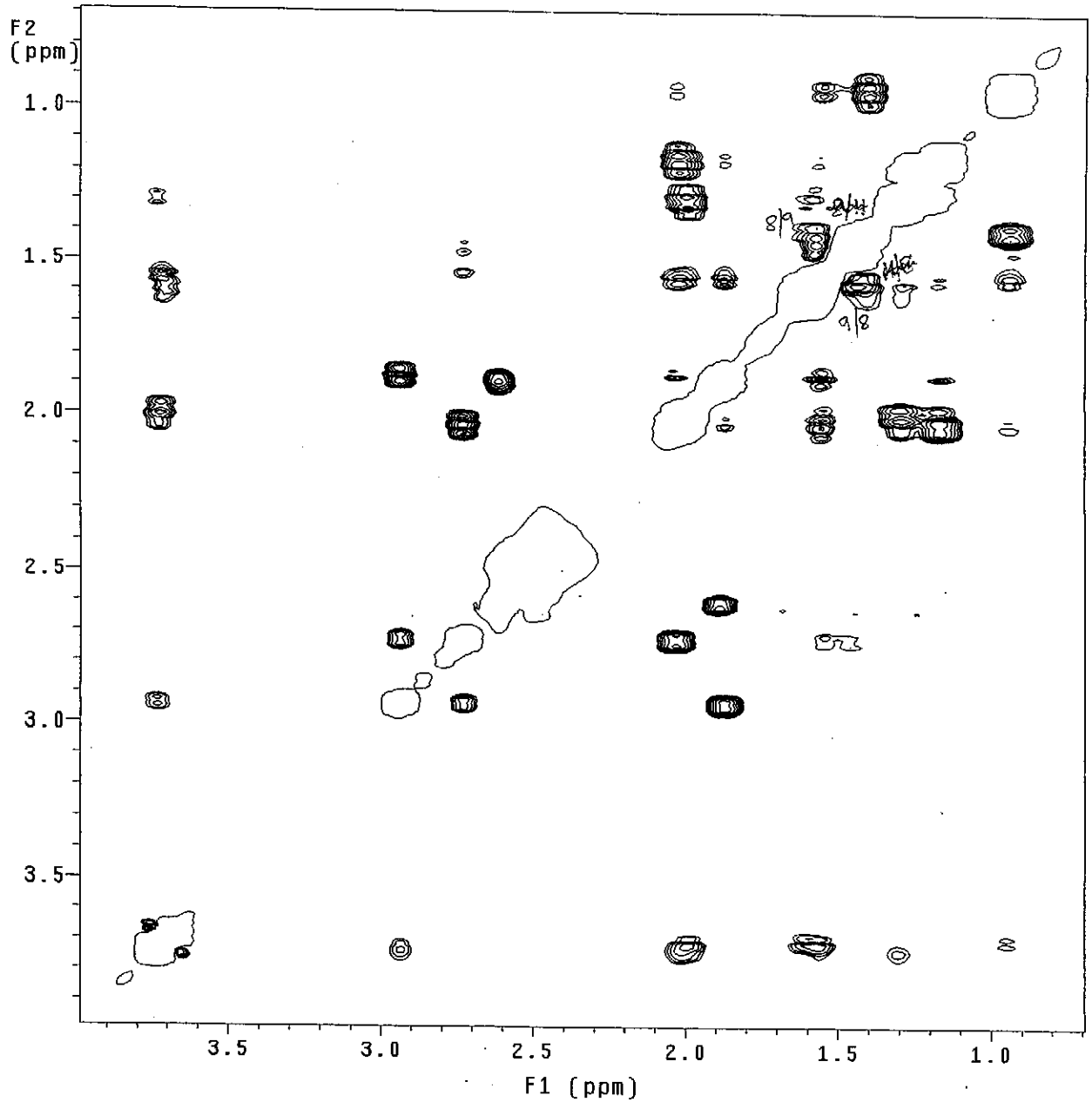


A-2

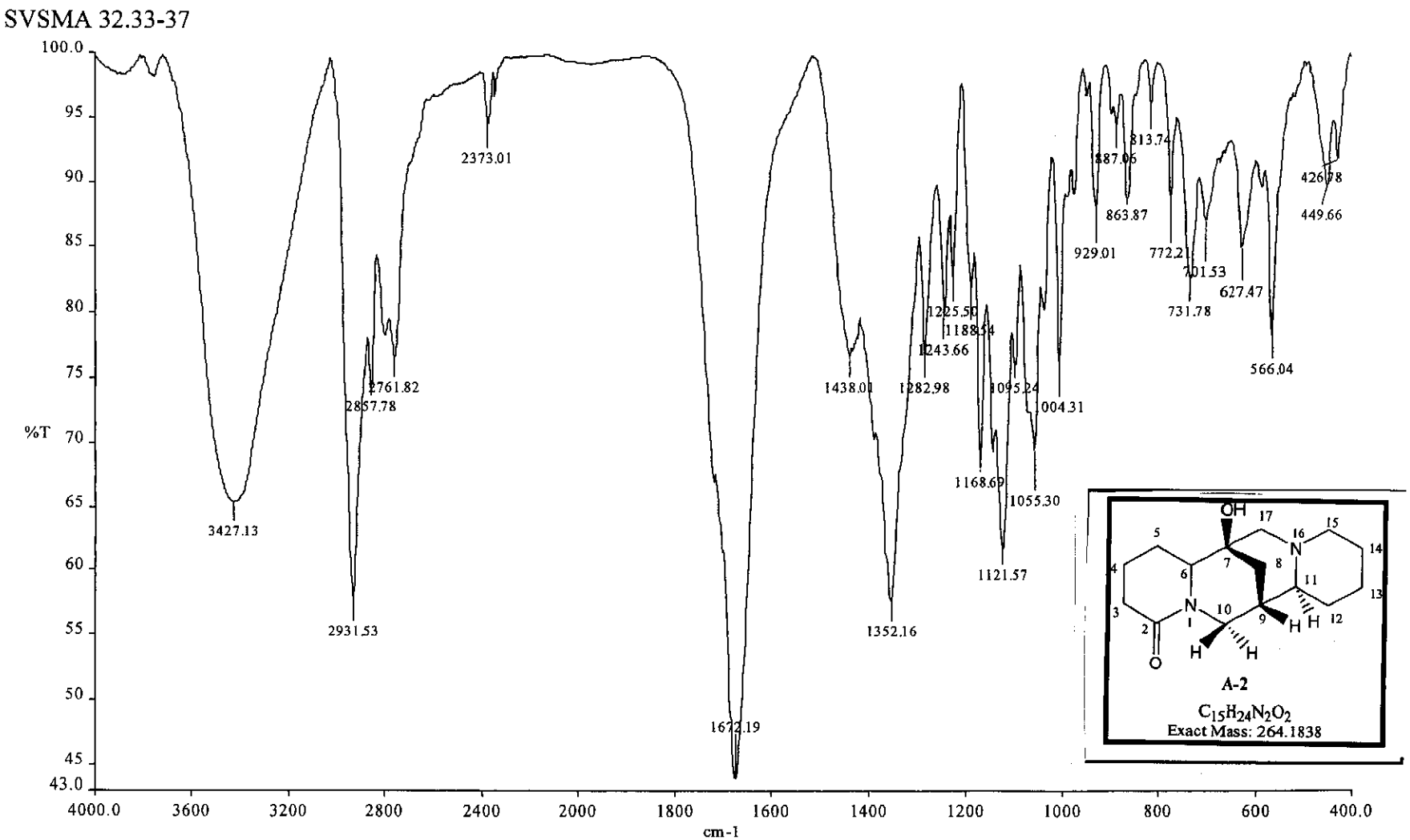
$C_{15}H_{24}N_2O_2$

Exact Mass: 264.1838

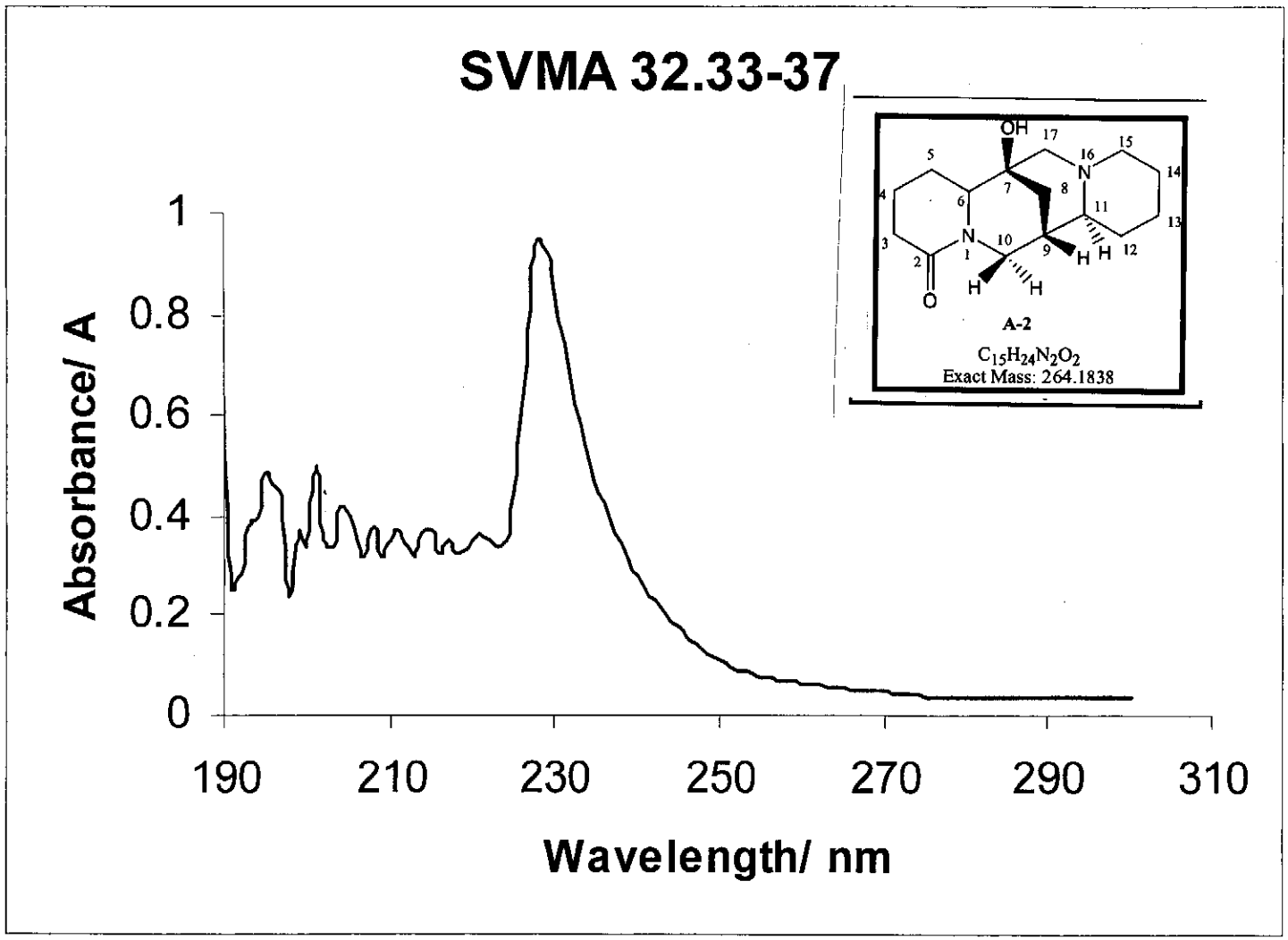




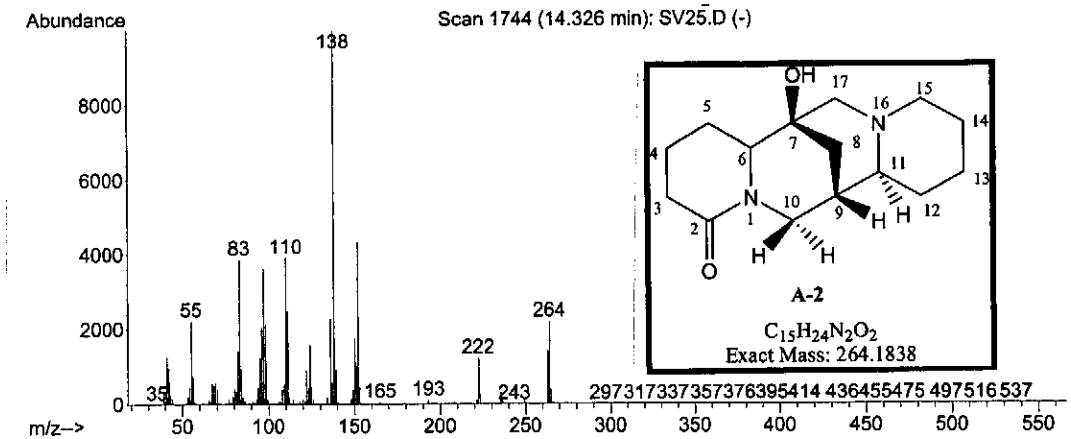
NOESY spectrum of -7-hydroxylupanine A2



IR spectrum of 7-hydroxylupanine A2



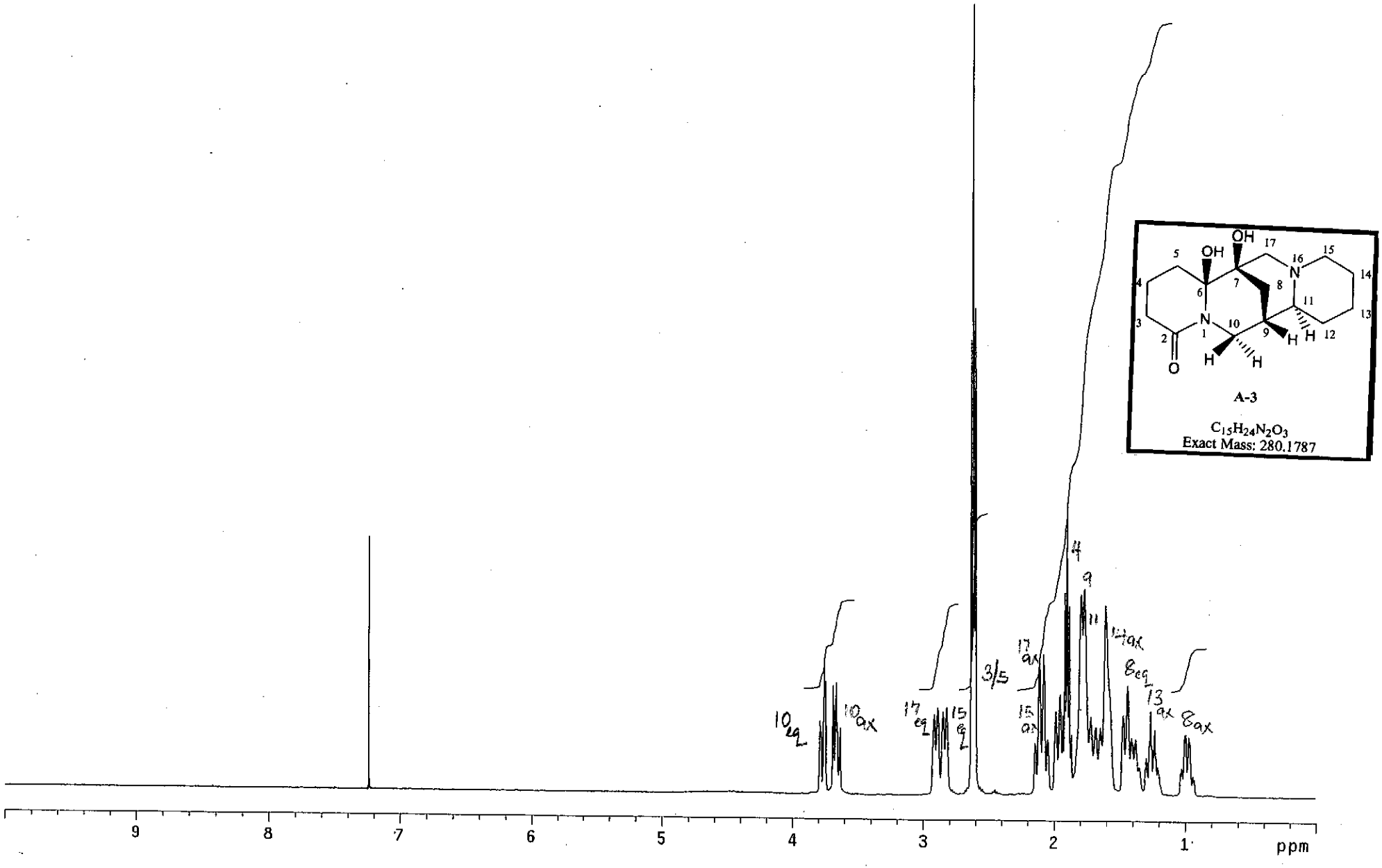
UV spectrum of 7-hydroxylupanine A2



Mass spectrum of 7-hydroxylupanine A2

hsv16.sv 25/30.36-40.16. in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

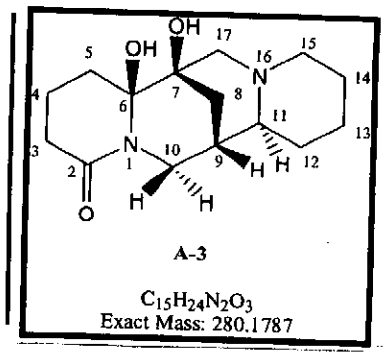


^{1}H NMR spectrum of 6,7-dihydroxylupanine A3

hsv16.sv 25/30.36-40.16 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2895.553	7.240	47.8	40	705.875	1.765	39.4
2	1515.307	3.789	13.9	41	686.097	1.716	15.2
3	1513.109	3.783	12.9	42	670.165	1.676	13.1
4	1502.671	3.757	25.7	43	657.346	1.644	13.1
5	1500.290	3.751	21.0	44	641.047	1.603	36.1
6	1498.276	3.746	21.5	45	630.426	1.576	20.5
7	1474.652	3.687	20.6	46	587.757	1.470	15.6
8	1467.144	3.668	18.2	47	574.388	1.436	21.0
9	1465.313	3.664	21.1	48	574.754	1.437	21.1
10	1461.833	3.655	13.5	49	561.752	1.405	11.2
11	1454.325	3.636	10.9	50	549.116	1.373	10.9
12	1452.494	3.632	12.5	51	517.069	1.293	7.5
13	1166.079	2.916	15.3	52	507.729	1.270	10.6
14	1155.091	2.888	16.8	53	504.250	1.261	16.4
15	1138.793	2.847	16.0	54	494.910	1.237	9.0
16	1127.256	2.819	16.8	55	491.431	1.229	12.8
17	1127.622	2.819	16.9	56	400.049	1.000	11.8
18	1053.455	2.634	62.5	57	396.570	0.992	11.9
19	1051.806	2.630	86.6	58	388.146	0.971	11.4
20	1045.397	2.614	150.0	59	383.934	0.960	10.5
21	1040.452	2.602	77.4				
22	1038.804	2.597	92.6				
23	856.957	2.143	10.0				
24	843.039	2.108	25.9				
25	830.769	2.077	26.9				
26	818.683	2.047	10.7				
27	816.302	2.041	10.4				
28	793.411	1.984	16.2				
29	780.592	1.952	19.3				
30	773.084	1.933	12.5				
31	771.435	1.929	15.4				
32	765.758	1.915	38.5				
33	765.026	1.913	38.6				
34	759.898	1.900	46.5				
35	758.433	1.896	54.1				
36	752.390	1.881	35.9				
37	746.896	1.868	10.7				
38	745.248	1.863	11.7				
39	716.497	1.792	38.2				



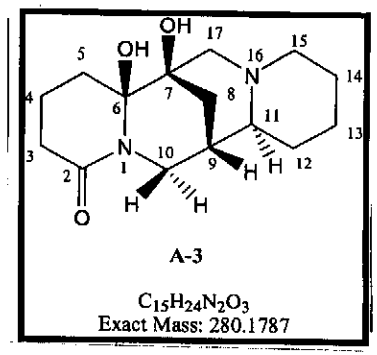
A-3

$C_{15}H_{24}N_2O_3$
Exact Mass: 280.1787

csv16.sv 25/30.36-40.16 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	17365.194	172.677	22.3
2	7775.515	77.319	28.5
3	7743.471	77.000	29.7
4	7711.426	76.681	29.3
5	6692.108	66.545	20.0
6	5690.338	56.584	14.3
7	5644.560	56.129	27.2
8	4147.628	41.243	35.7
9	3933.235	39.112	13.6
10	3306.080	32.875	100.0
11	2914.680	28.983	21.8
12	2784.976	27.693	34.2
13	2501.154	24.871	21.8
14	2421.806	24.082	32.5
15	1719.117	17.095	50.6



A-3

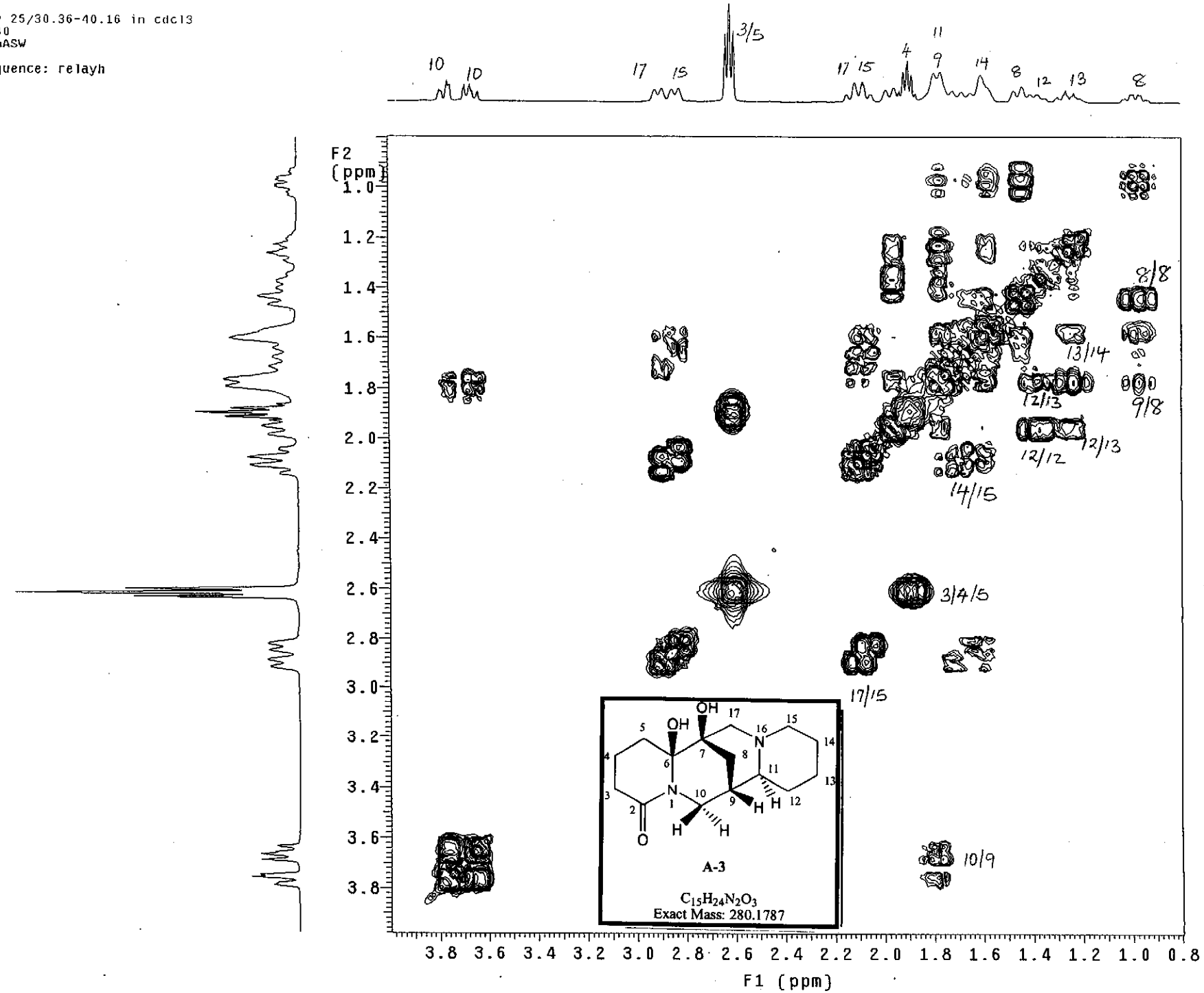
$C_{15}H_{24}N_2O_3$
Exact Mass: 280.1787

* C-6 and C-7 could not be detected.

^{13}C NMR spectrum of 6,7-dihydroxylupanine A 3

cysv16.sv 25/30.36-40.16 in cdcl3
1H Cosy-90
probe=5mmASW

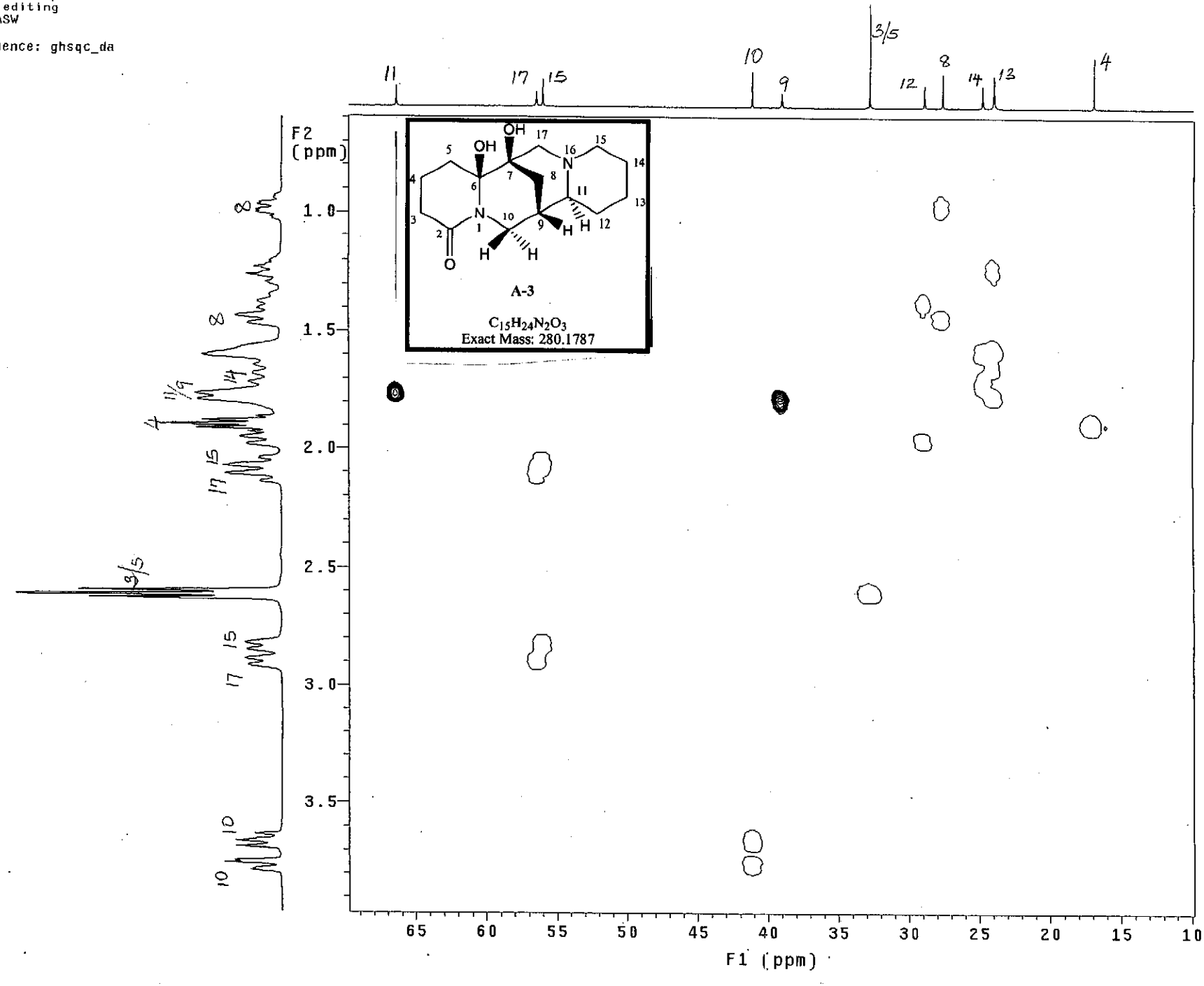
Pulse Sequence: relayh



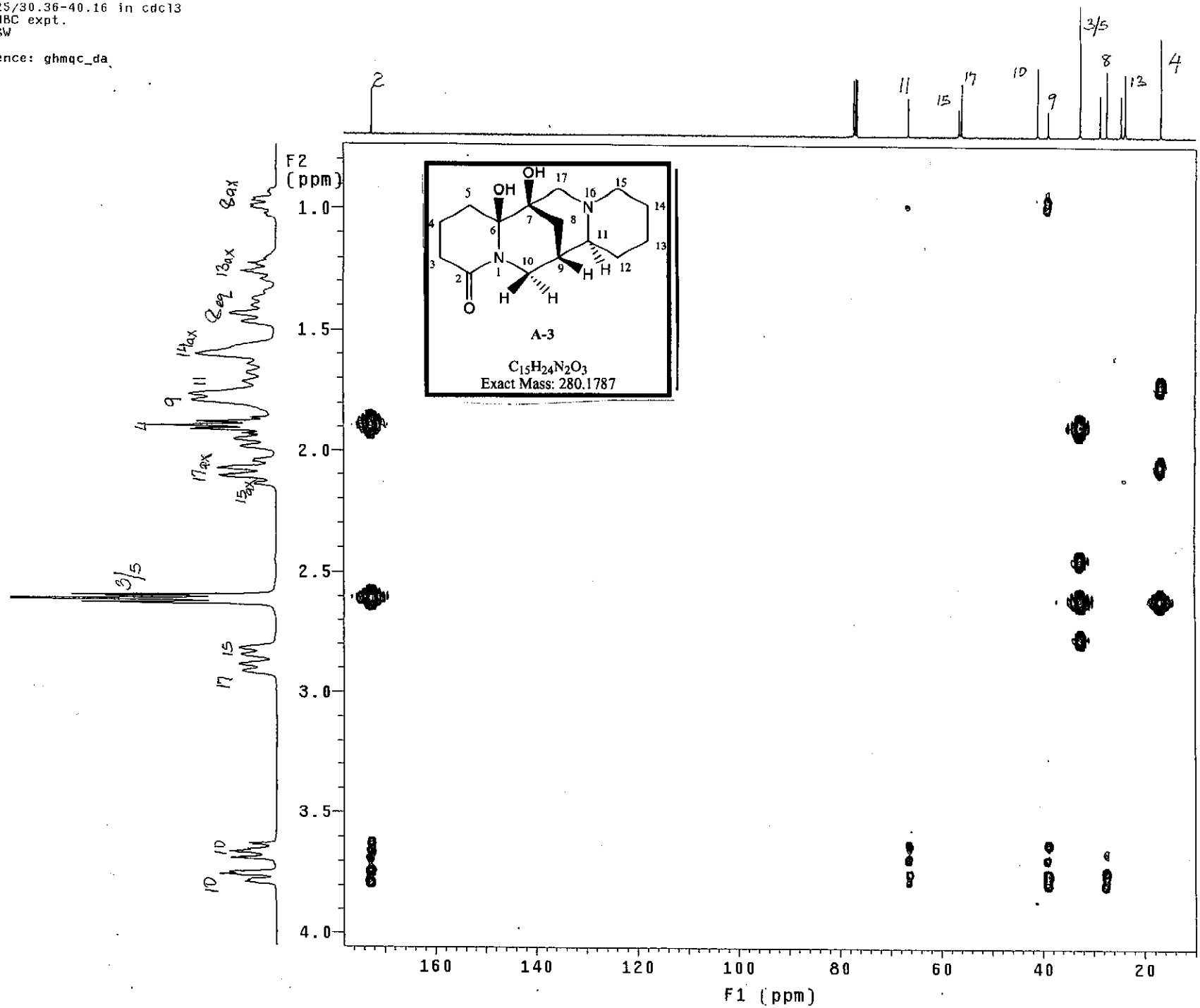
COSY spectrum of 6,7-dihydroxylupanine A3

HQsv16.sv 25/30.36-40.16 in cdcl3
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da



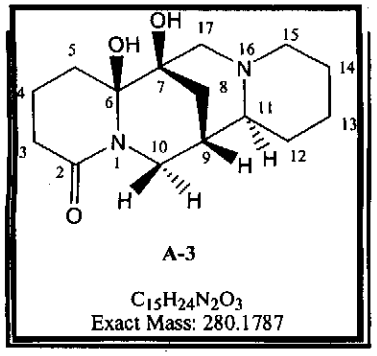
HSQC spectrum of 6,7-dihydroxylupanine A3



HMBC spectrum of 6,7-dihydroxylupanine A3

NOsv16.sv 25/30.36-40.16 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW

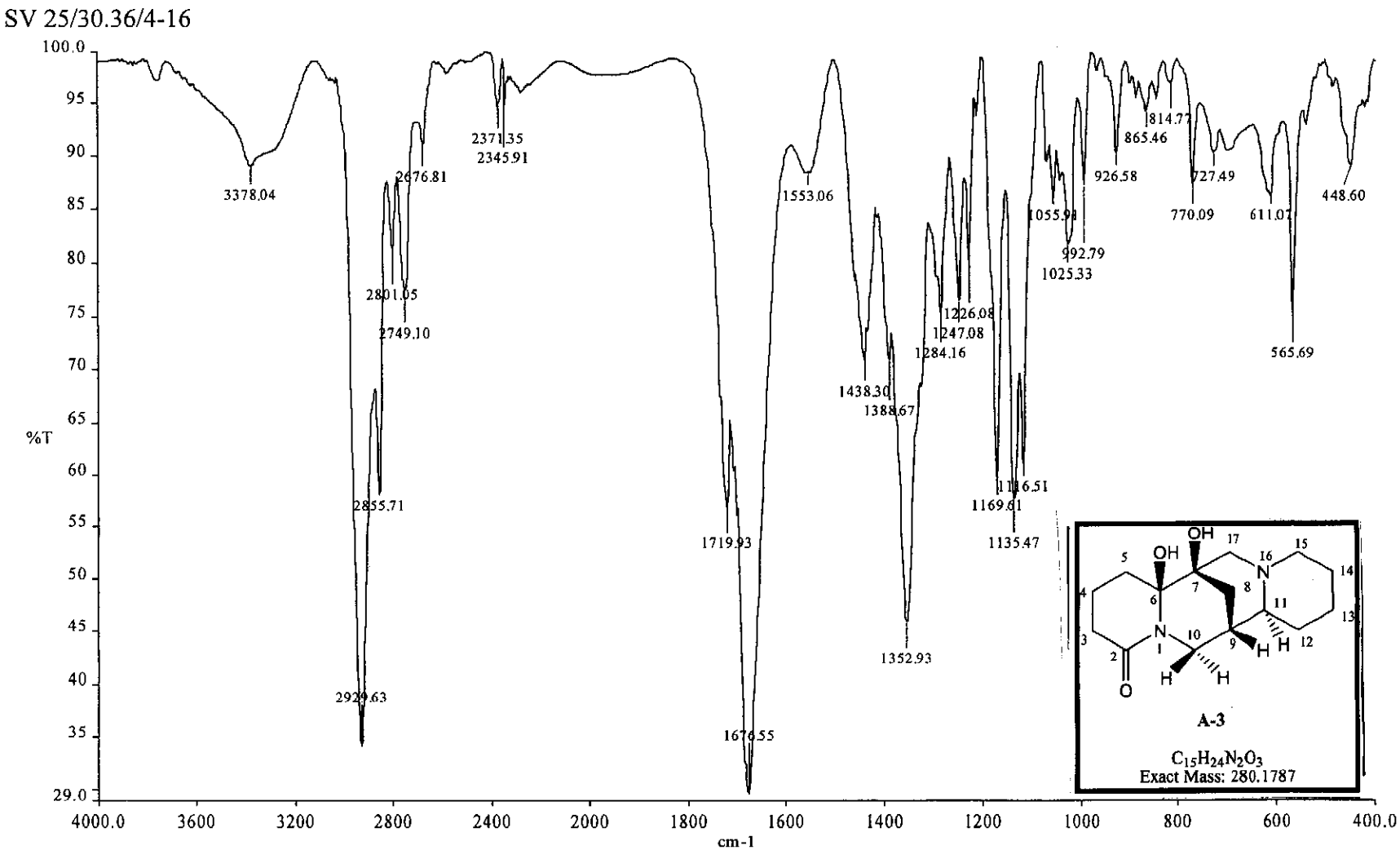
Pulse Sequence: noesy_da



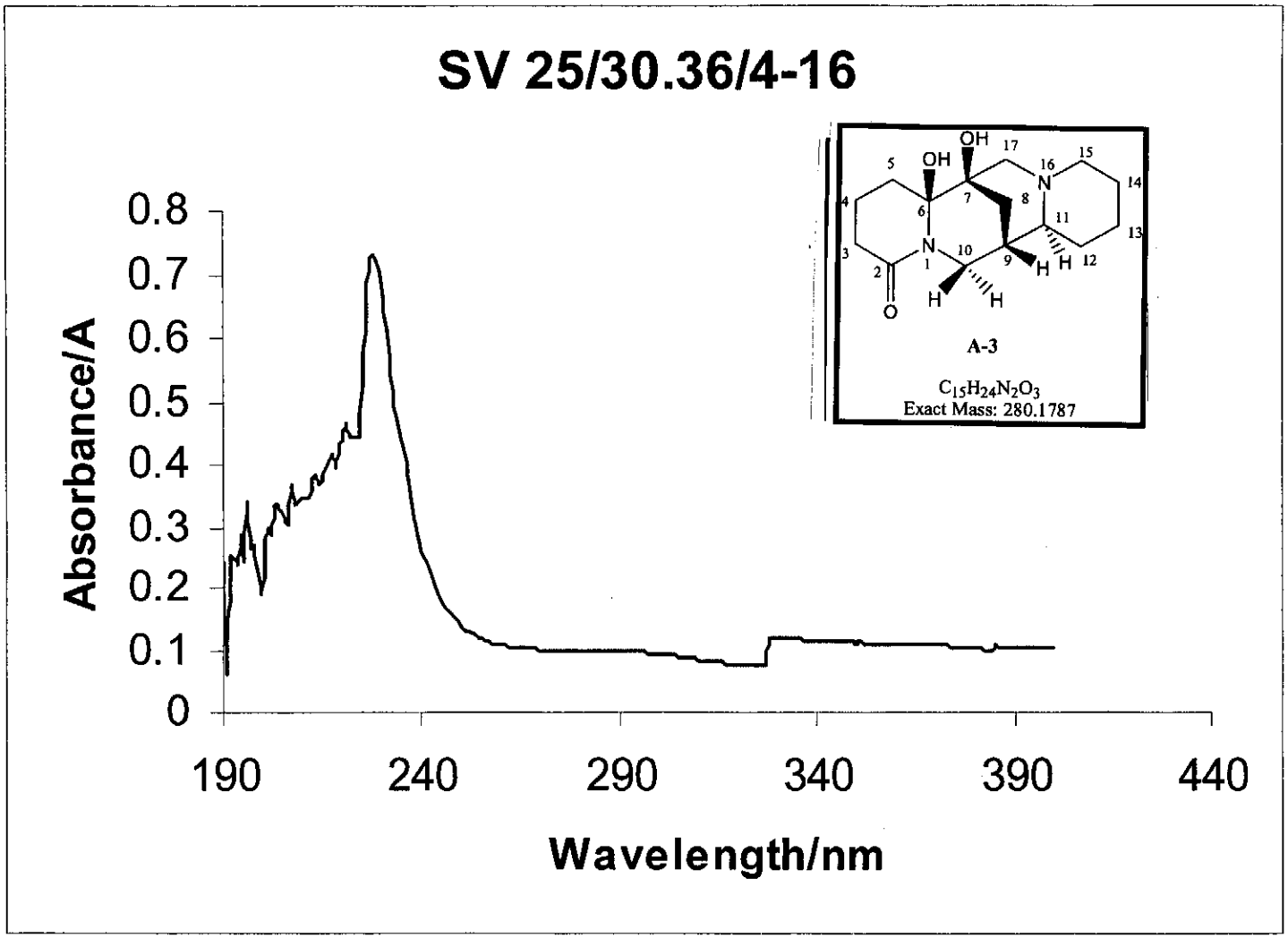
A-3

$C_{15}H_{24}N_2O_3$
Exact Mass: 280.1787

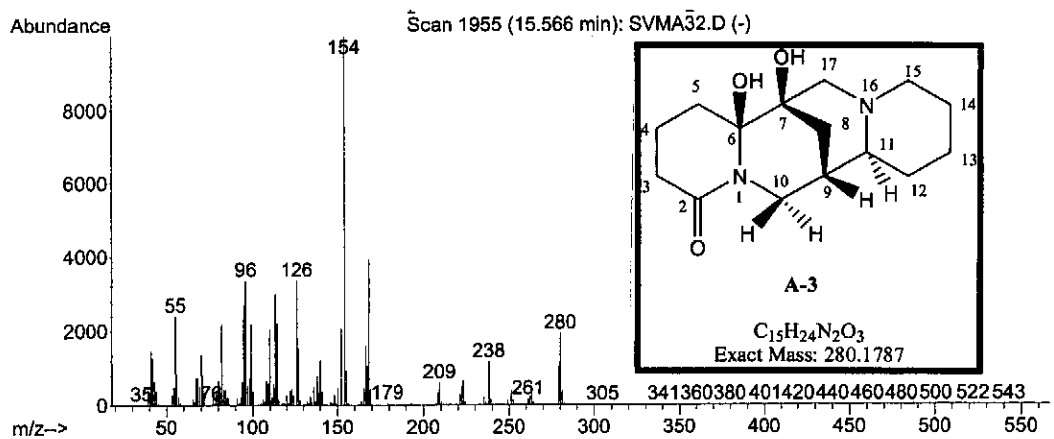
NOESY spectrum of 6,7-dihydroxylupanine A3



IR spectrum of 6,7-dihydroxylupanine A3



UV spectrum of 6,7-dihydroxylupanine A3



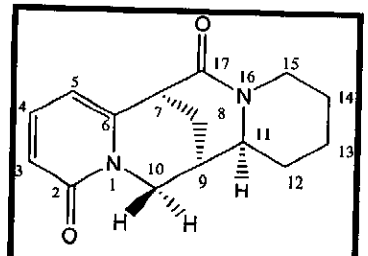
Mass spectrum of 6,7-dihydroxylupanine A3

^{1}H NMR spectrum of 17-oxo-thermopsine A4

hsv412.svdcmx 4.1.2 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2910.203	7.277	11.9	40	934.970	2.338	10.5
2	2903.428	7.260	12.6	41	919.770	2.300	8.7
3	2901.047	7.254	13.4	42	918.122	2.296	5.5
4	2897.018	7.244	6.8	43	805.131	2.013	5.4
5	2895.919	7.241	250.0	44	802.018	2.005	10.3
6	2894.454	7.237	24.3	45	799.088	1.998	7.0
7	2589.178	6.474	11.9	46	791.580	1.979	6.1
8	2587.896	6.471	14.1	47	788.833	1.972	10.3
9	2580.204	6.452	11.8	48	785.536	1.964	8.2
10	2578.739	6.448	13.2	49	780.409	1.951	5.4
11	2510.798	6.278	10.5	50	774.549	1.937	5.9
12	2509.516	6.275	10.9	51	692.140	1.731	6.3
13	2504.022	6.261	11.1	52	683.716	1.710	7.4
14	2502.557	6.257	10.7	53	664.854	1.662	7.4
15	1833.586	4.585	5.4	54	649.838	1.625	15.2
16	1831.388	4.579	5.2	55	646.724	1.617	17.6
17	1822.232	4.556	5.4	56	643.428	1.609	19.3
18	1820.400	4.552	5.8	57	637.568	1.594	15.8
19	1818.203	4.546	5.6	58	633.905	1.585	20.7
20	1697.887	4.245	10.1	59	630.609	1.577	19.0
21	1682.138	4.206	13.5	60	625.481	1.564	12.0
22	1574.458	3.937	11.9	61	622.002	1.555	14.1
23	1568.048	3.921	12.5	62	613.395	1.534	6.2
24	1558.709	3.897	9.7	63	609.732	1.525	5.4
25	1552.299	3.881	9.9	64	565.232	1.413	5.7
26	1448.098	3.621	6.8	65	553.145	1.383	5.3
27	1445.352	3.614	11.2	66	491.614	1.229	71.1
28	1442.788	3.608	12.1	67	342.546	0.856	11.0
29	1439.858	3.600	5.9	68	335.404	0.839	5.4
30	1440.224	3.601	6.0	69	331.559	0.829	5.2
31	1332.544	3.332	5.2				
32	1323.754	3.310	6.5				
33	975.991	2.440	7.1				
34	972.328	2.431	6.2				
35	963.721	2.410	5.9				
36	960.974	2.403	5.7				
37	950.719	2.377	9.2				
38	947.972	2.370	10.6				
39	937.717	2.345	7.9				



A-4

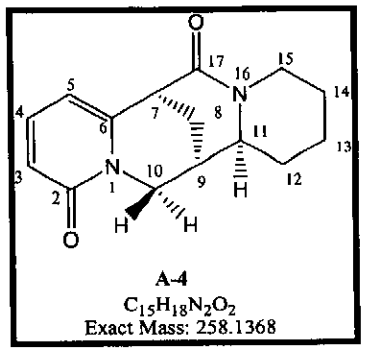
$C_{15}H_{18}N_2O_2$

Exact Mass: 258.1368

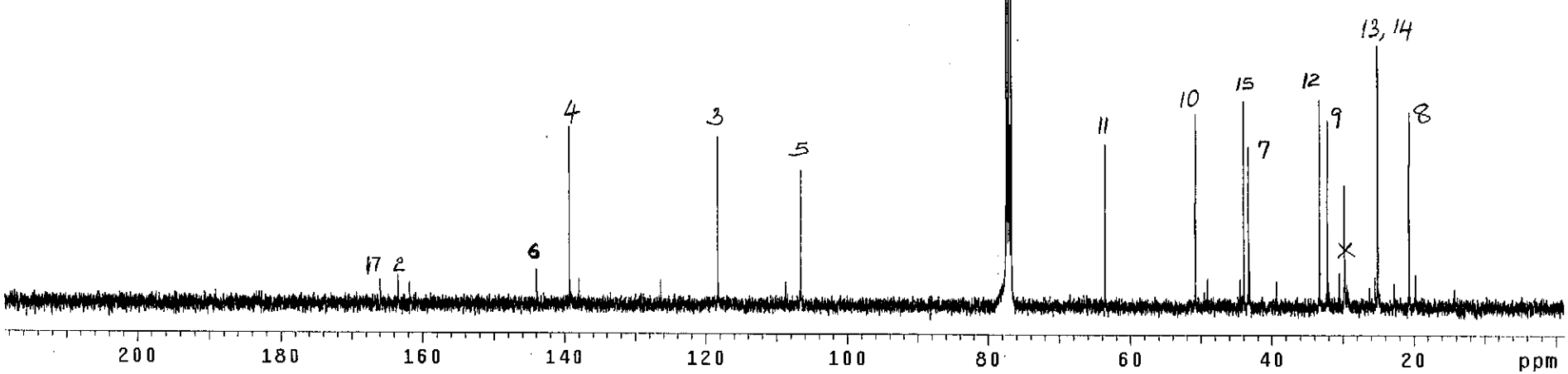
csv412.svdcmx 4.1.2 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

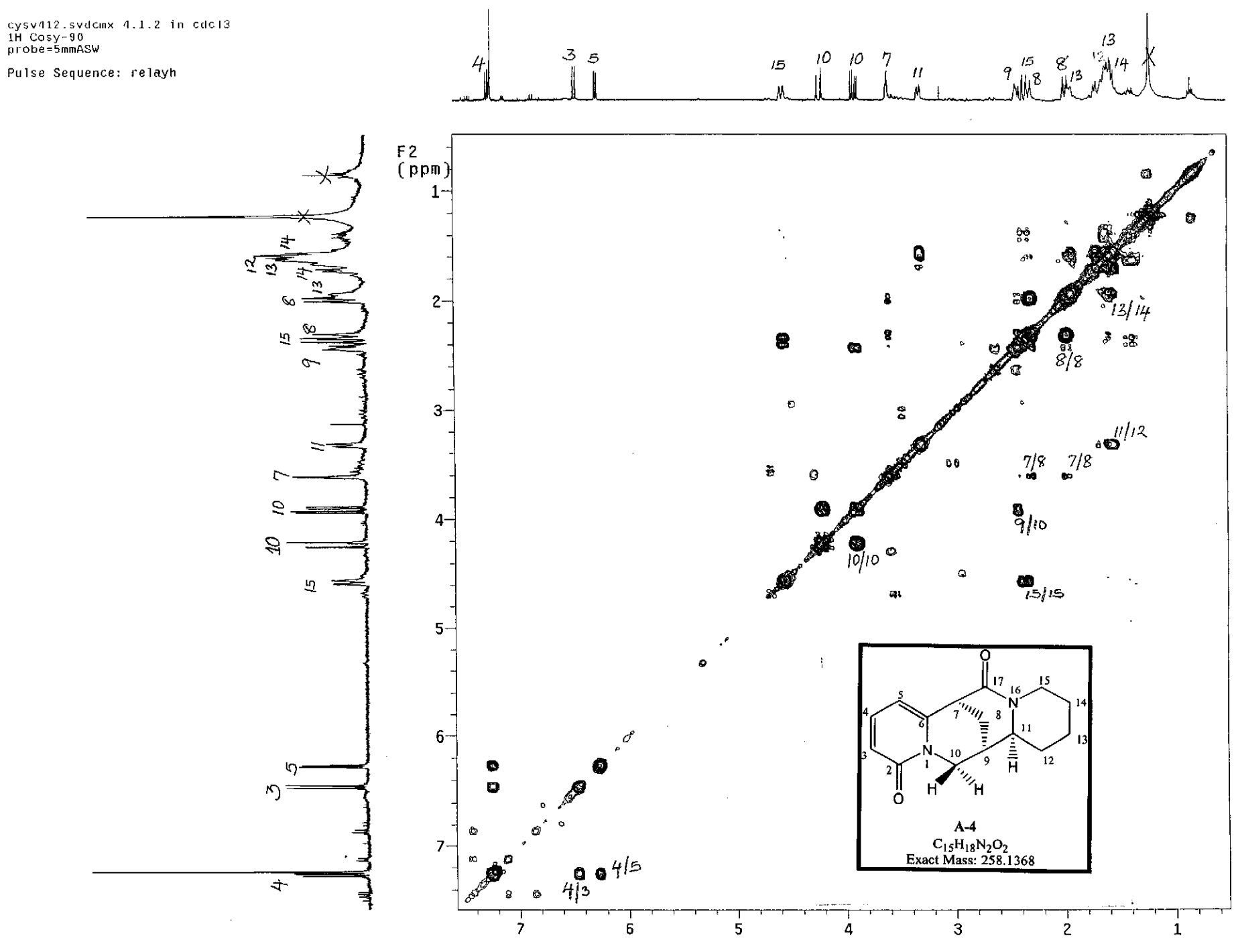
INDEX	FREQUENCY	PPM	HEIGHT
1	16690.735	165.970	4.0
2	16439.720	163.474	4.6
3	16271.868	161.805	3.7
4	14476.617	143.953	5.7
5	14005.106	139.265	28.4
6	13873.113	137.952	4.4
7	12710.358	126.390	4.1
8	11889.410	118.227	27.1
9	10929.603	108.682	3.9
10	10715.210	106.551	21.9
11	7775.515	77.319	384.6
12	7764.071	77.205	24.1
13	7743.471	77.000	400.0
14	7711.426	76.681	392.7
15	6385.397	63.496	26.2
16	5097.516	50.689	31.0
17	4925.086	48.974	4.7
18	4460.442	44.354	4.6
19	4410.086	43.853	33.0
20	4340.657	43.163	25.9
21	4324.635	43.004	5.9
22	3948.494	39.263	4.3
23	3336.598	33.179	33.4
24	3227.494	32.094	30.0
25	3209.183	31.912	4.2
26	3057.353	30.402	5.7
27	2985.635	29.689	19.7
28	2552.272	25.379	5.1
29	2512.598	24.985	41.8
30	2509.546	24.955	40.6
31	2280.657	22.679	4.1
32	2067.028	20.554	31.3
33	1973.183	19.621	5.4



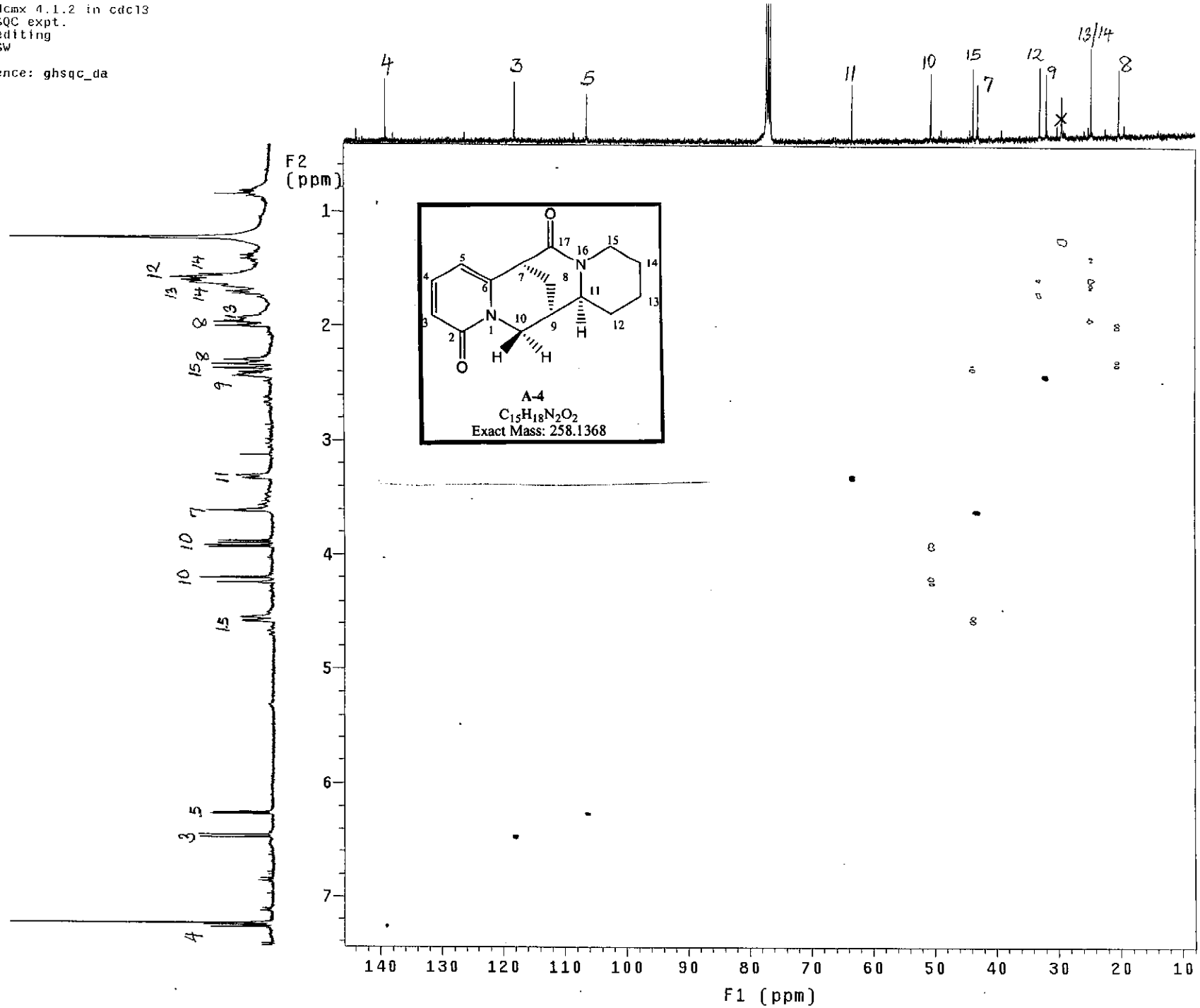
A-4
$C_{15}H_{18}N_2O_2$
Exact Mass: 258.1368



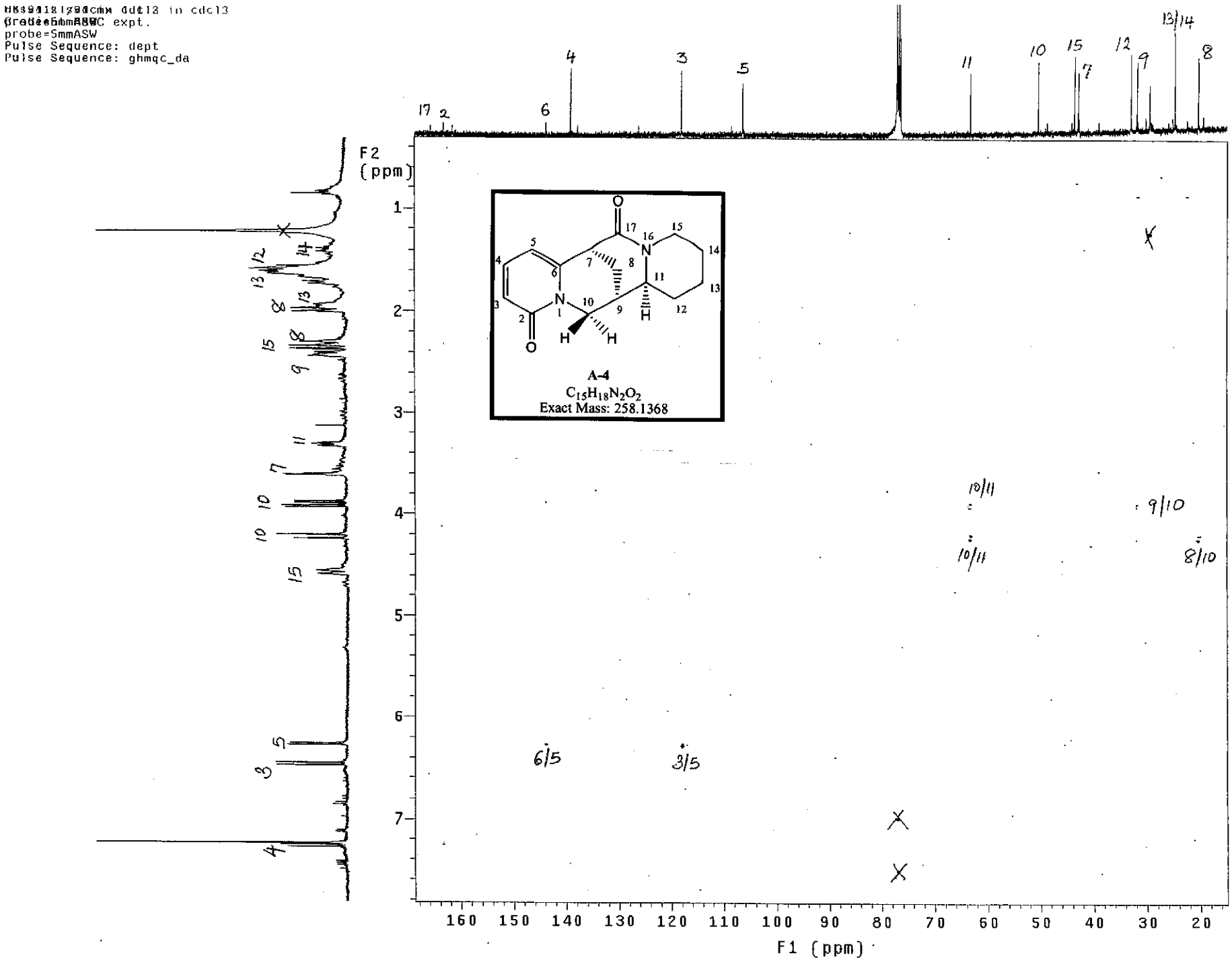
^{13}C NMR spectrum of 17-oxo-thermopsine A4



COSY spectrum of 17-oxo-thermopsine A4



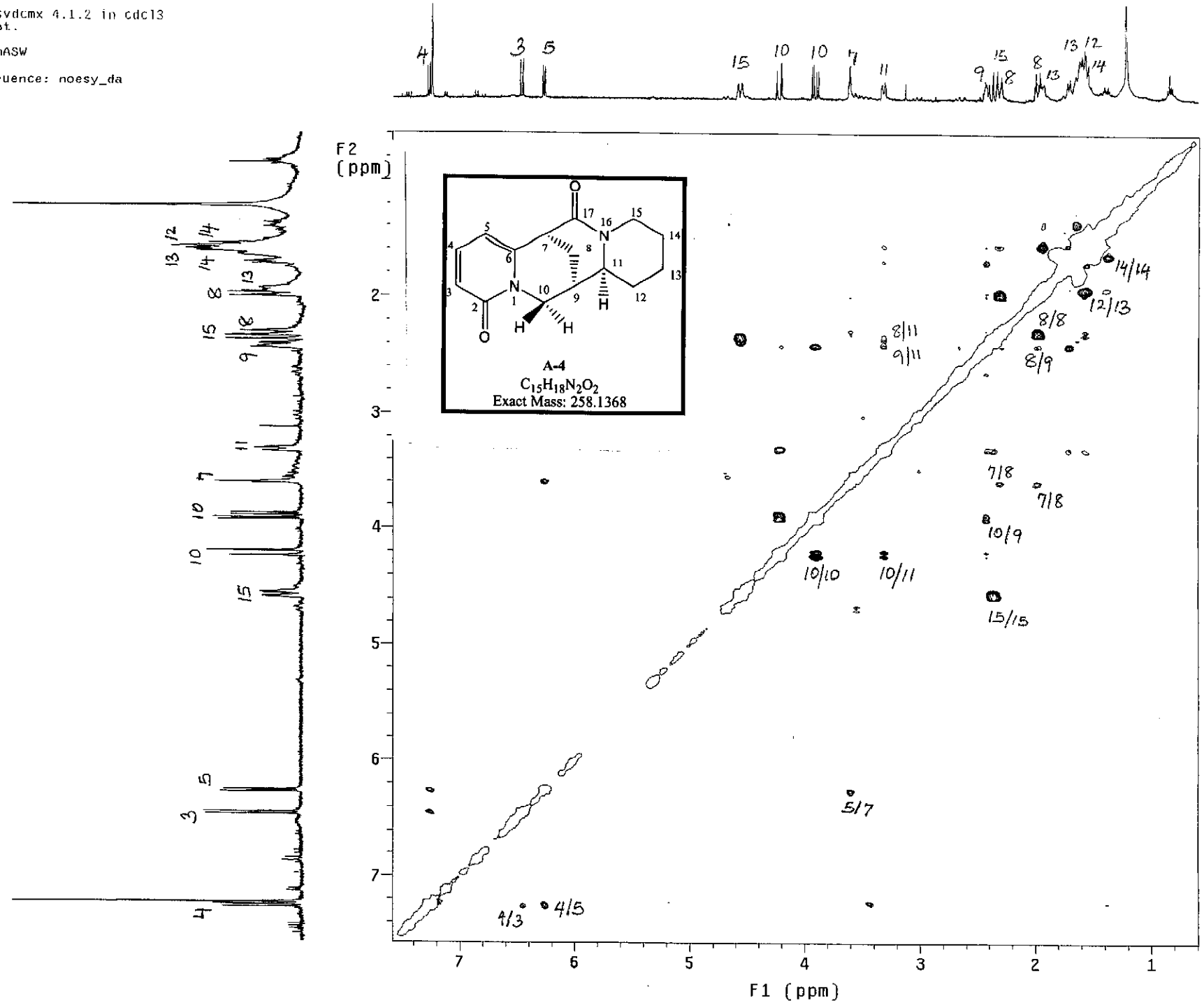
HSQC spectrum of 17-oxo-thermopsine A4



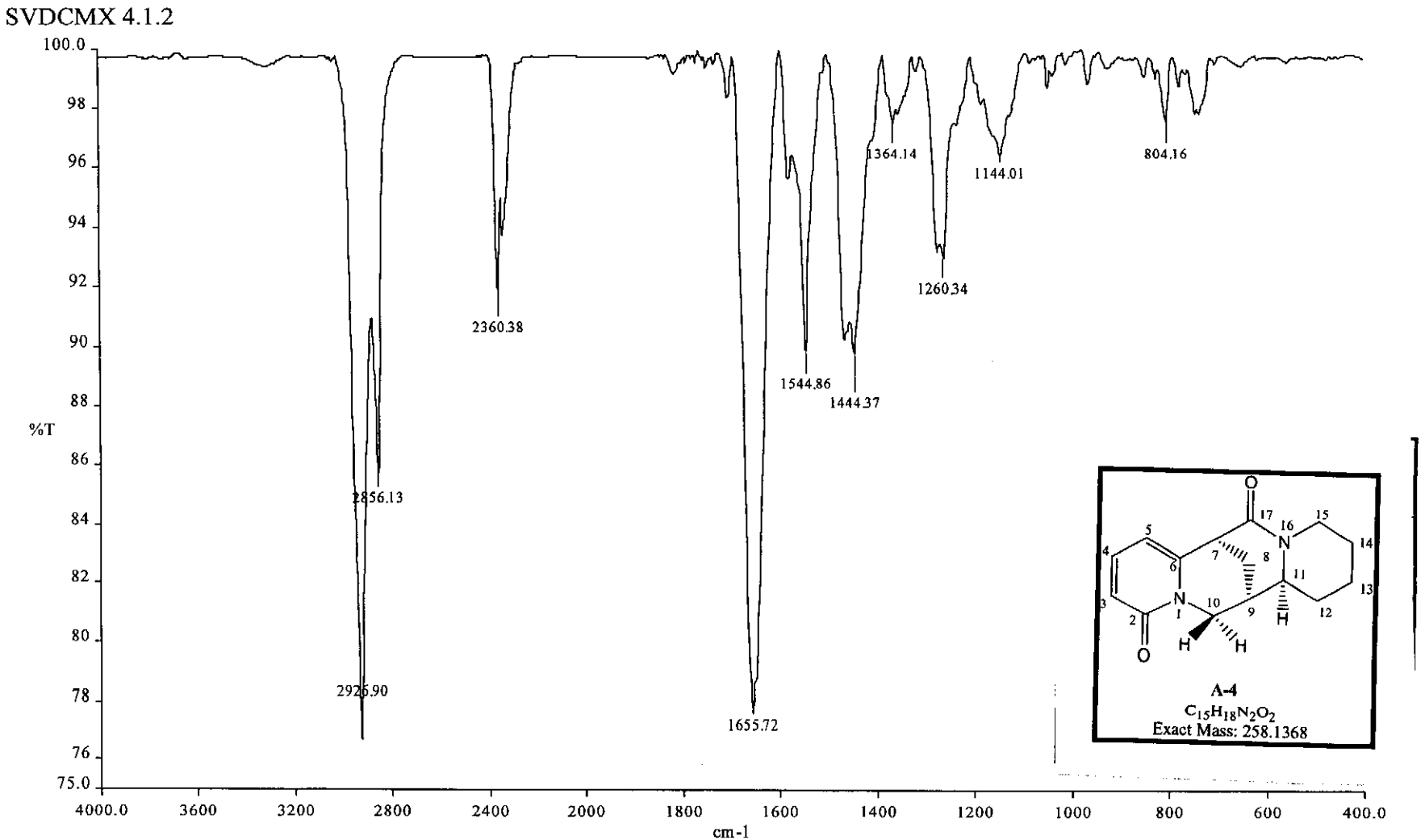
HMBC spectrum of 17-oxo-thermopsine A4

NOsv412.svdcmx 4.1.2 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW

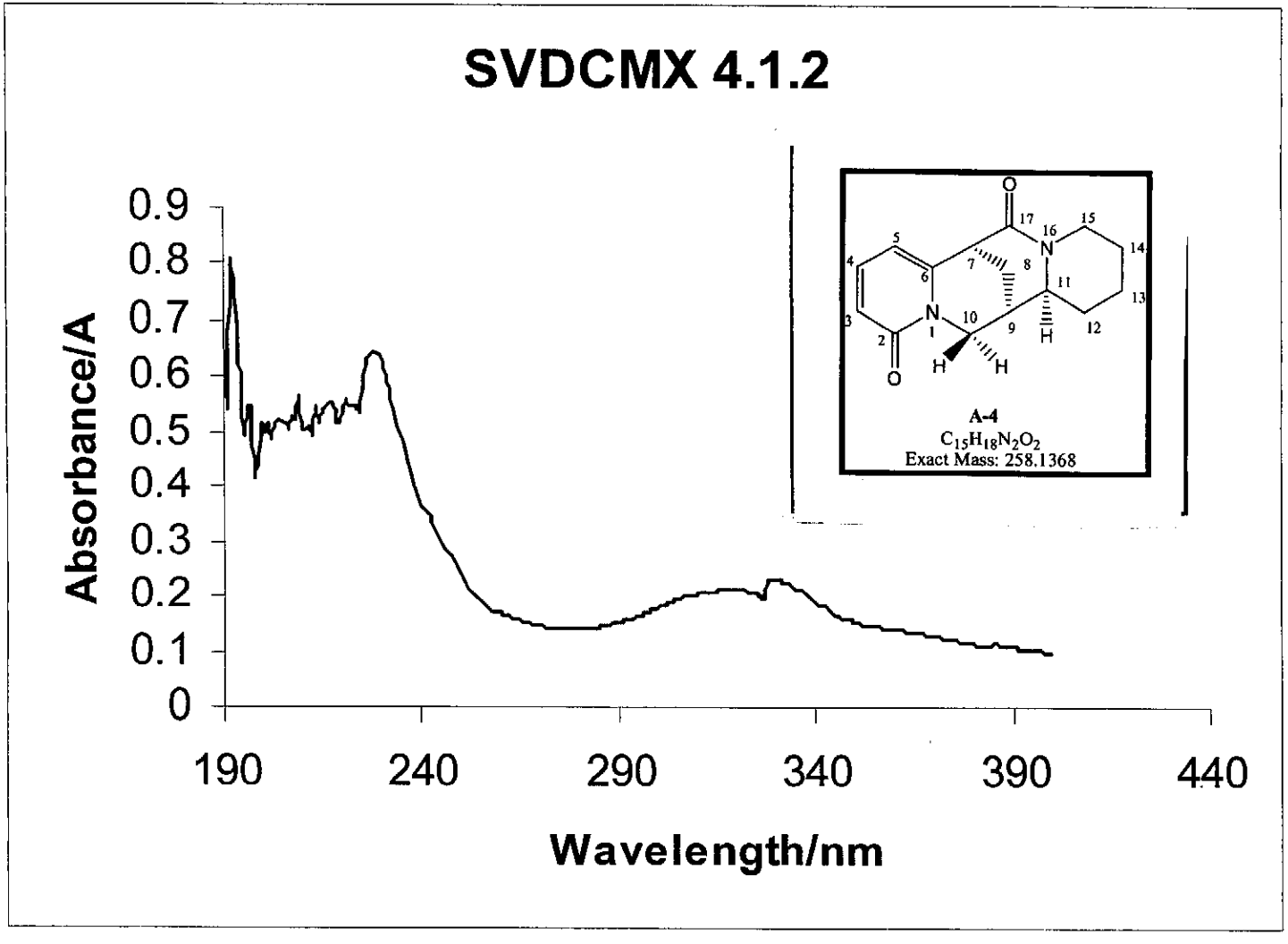
Pulse Sequence: noesy_da



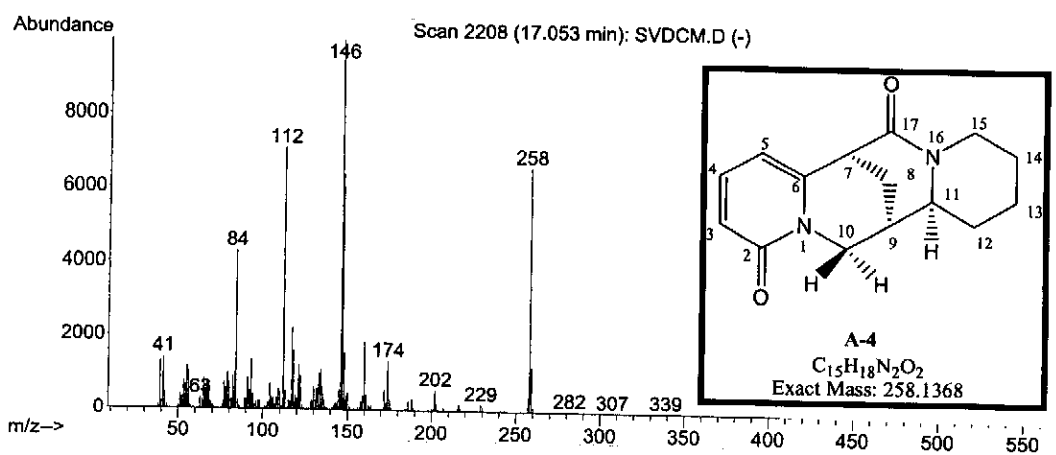
NOESY spectrum of 17-oxo-thermopsine A4



IR spectrum of 17-oxo-thermopsine A4



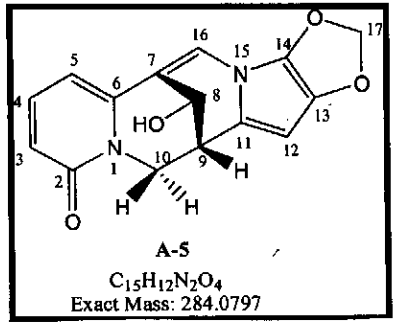
UV spectrum of 17-oxo-thermopsine A4



Mass spectrum of 17-oxo-thermopsine A4

hsv31.svsd 12-14/2/5/31 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul



A-5

$C_{15}H_{12}N_2O_4$

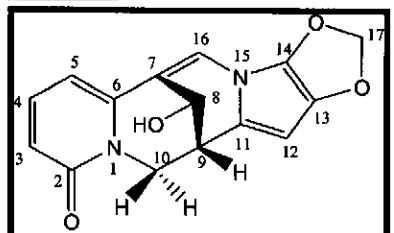
Exact Mass: 284.0797

^{1}H NMR spectrum of velutinine A5

hsv31.svsd 12-14/2/5/31 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2952.323	7.382	3.3	40	1391.878	3.480	5.4
2	2943.899	7.361	4.2	41	1386.750	3.467	4.8
3	2941.885	7.356	15.8	42	1384.736	3.462	6.1
4	2933.461	7.335	16.5	43	1379.974	3.450	5.7
5	2897.201	7.244	4.3	44	1375.396	3.439	3.3
6	2895.553	7.240	150.0	45	1375.762	3.440	3.4
7	2895.187	7.239	129.9	46	1374.114	3.436	3.7
8	2894.088	7.236	6.3	47	1368.987	3.423	3.1
9	2839.332	7.099	3.2	48	623.101	1.558	68.9
10	2678.911	6.698	25.1	49	492.529	1.232	13.9
11	2615.914	6.541	12.6				
12	2613.351	6.534	11.5				
13	2607.490	6.520	12.2				
14	2604.927	6.513	11.4				
15	2575.626	6.440	3.5				
16	2572.147	6.431	3.4				
17	2569.400	6.424	5.2				
18	2564.821	6.413	23.0				
19	2558.229	6.397	16.6				
20	2555.848	6.391	15.1				
21	2360.998	5.903	23.1				
22	2359.533	5.900	28.4				
23	2349.827	5.875	28.1				
24	2348.729	5.873	23.3				
25	2182.813	5.458	11.6				
26	2175.855	5.440	11.9				
27	1973.868	4.935	9.3				
28	1687.815	4.220	9.1				
29	1682.870	4.208	9.7				
30	1676.827	4.193	9.6				
31	1671.699	4.180	9.7				
32	1582.332	3.956	3.6				
33	1498.825	3.748	30.9				
34	1458.354	3.646	10.5				
35	1447.366	3.619	21.8				
36	1439.675	3.600	5.0				
37	1436.378	3.591	12.9				
38	1428.687	3.572	3.4				
39	1429.053	3.573	3.5				



A-5

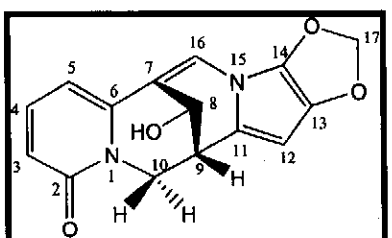
$C_{15}H_{12}N_2O_4$

Exact Mass: 284.0797

csv31.svsd 12-14/2/5/31 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	15786.624	156.980	8.4
2	15750.765	156.623	6.5
3	15506.616	154.196	6.6
4	14891.668	148.081	9.5
5	14250.017	141.700	7.9
6	13294.787	132.202	4.8
7	13284.869	132.103	28.0
8	12544.795	124.744	5.0
9	11855.077	117.885	13.6
10	11327.870	112.643	7.4
11	11034.892	109.729	29.9
12	10696.899	106.368	4.6
13	10531.336	104.722	30.0
14	10422.996	103.645	31.7
15	10184.951	101.278	39.0
16	9434.959	93.820	31.3
17	7886.145	78.419	31.0
18	7775.515	77.319	192.7
19	7764.071	77.205	11.6
20	7743.471	77.000	200.0
21	7711.426	76.681	194.7
22	6689.819	66.523	4.9
23	6680.664	66.432	37.3
24	4033.946	40.113	26.9



A-5

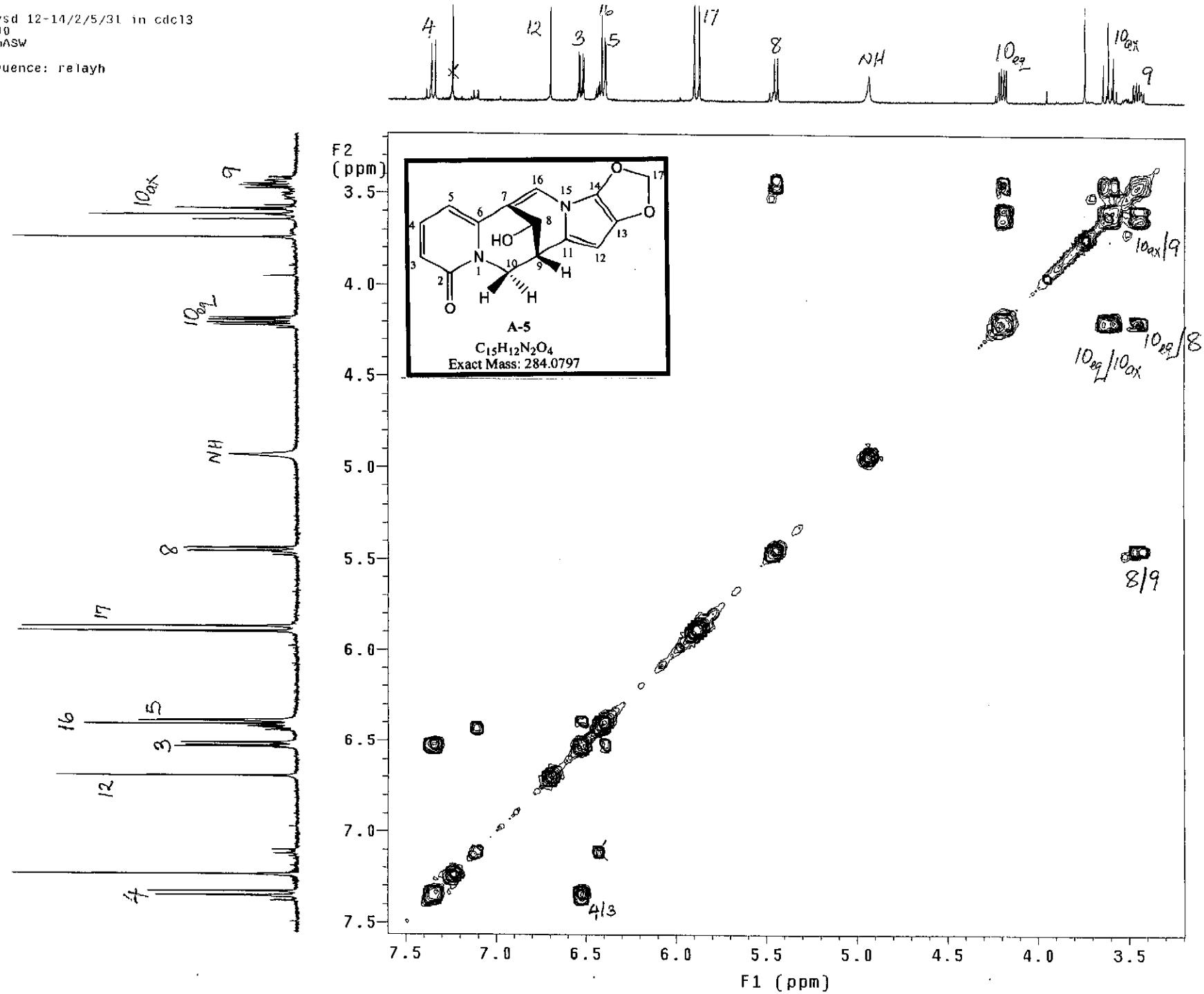
$C_{15}H_{12}N_2O_4$

Exact Mass: 284.0797

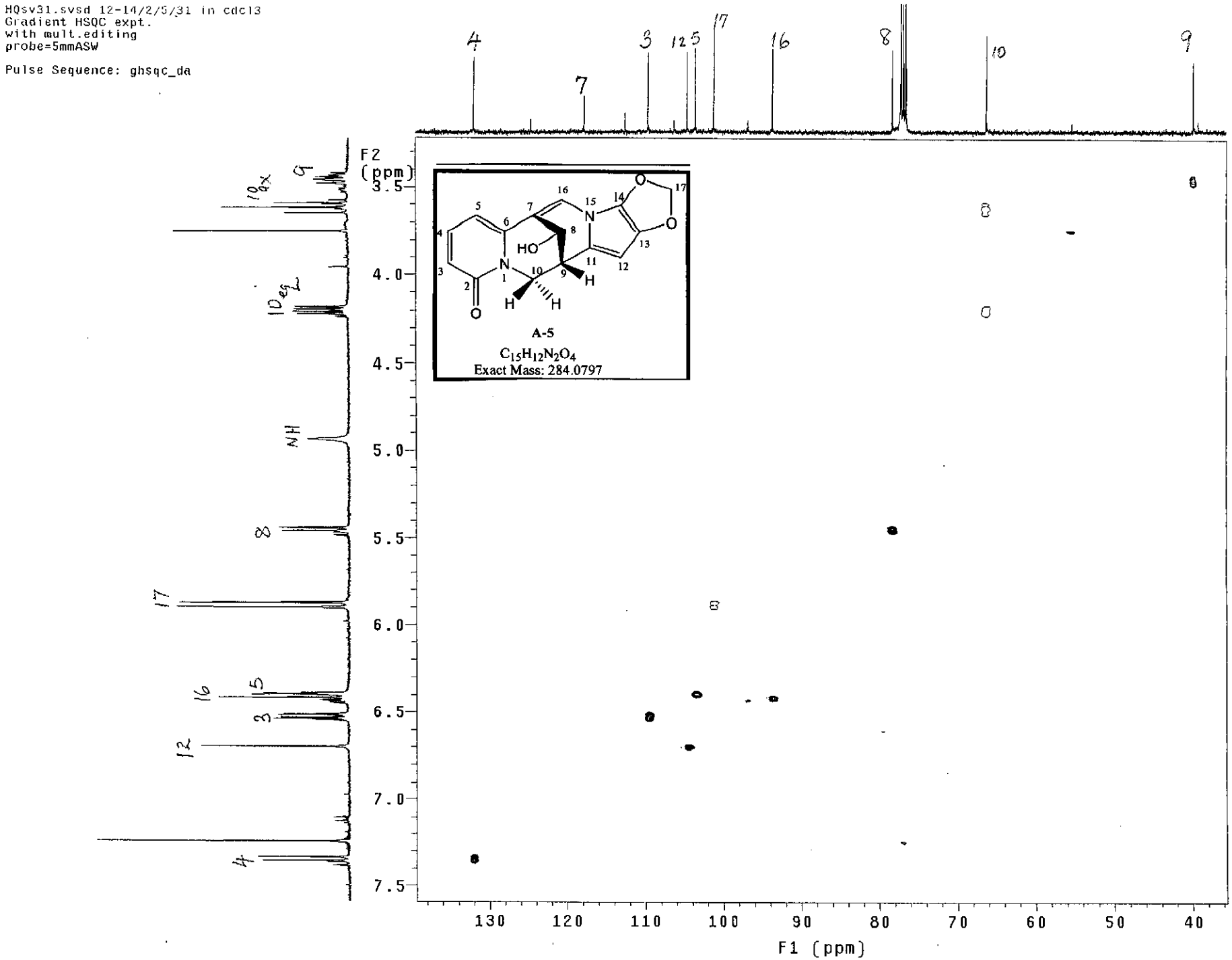
^{13}C NMR spectrum of velutinine A5

cysv31.svsd 12-14/2/5/31 in cdcl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh



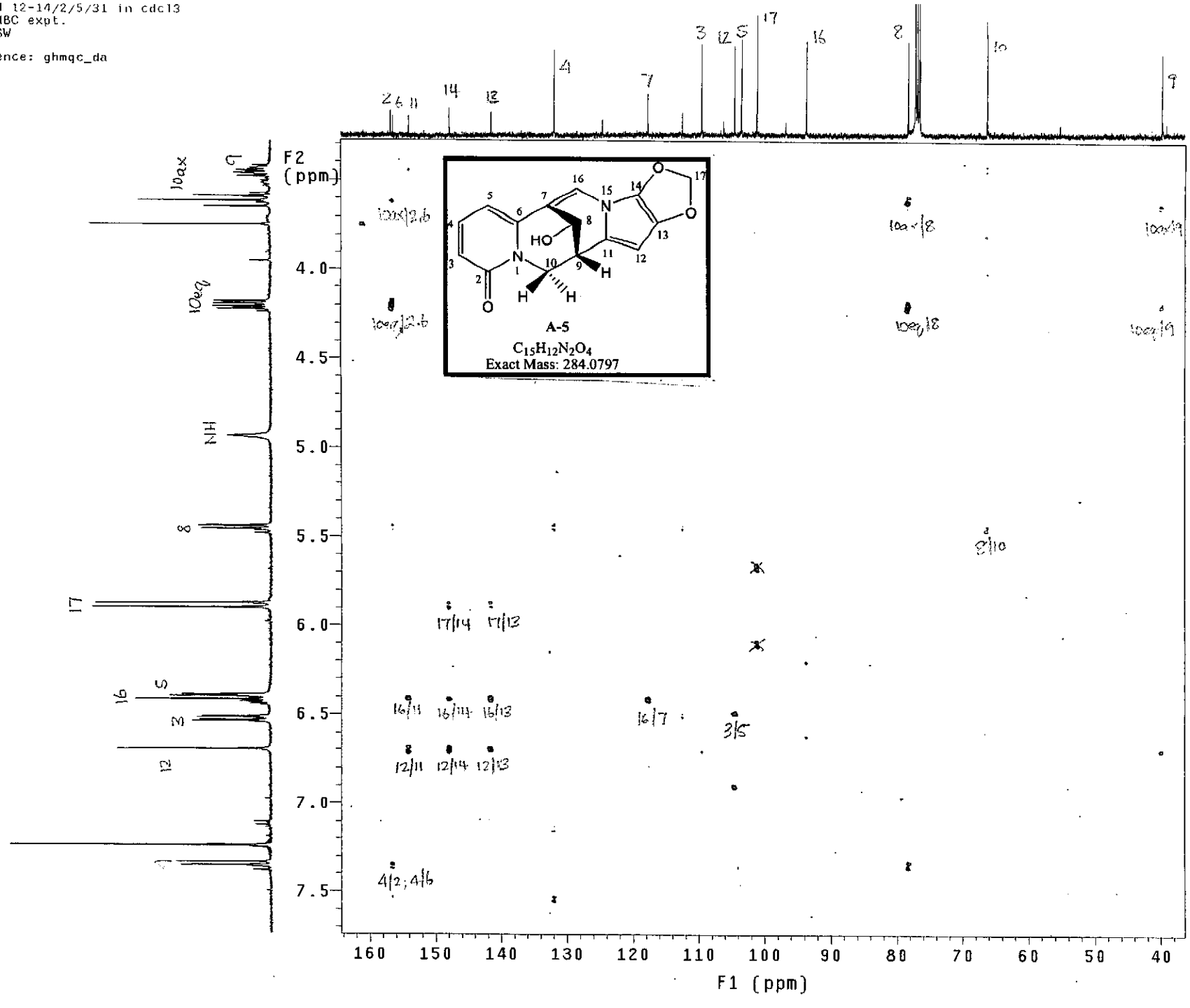
COSY spectrum of velutinine A5



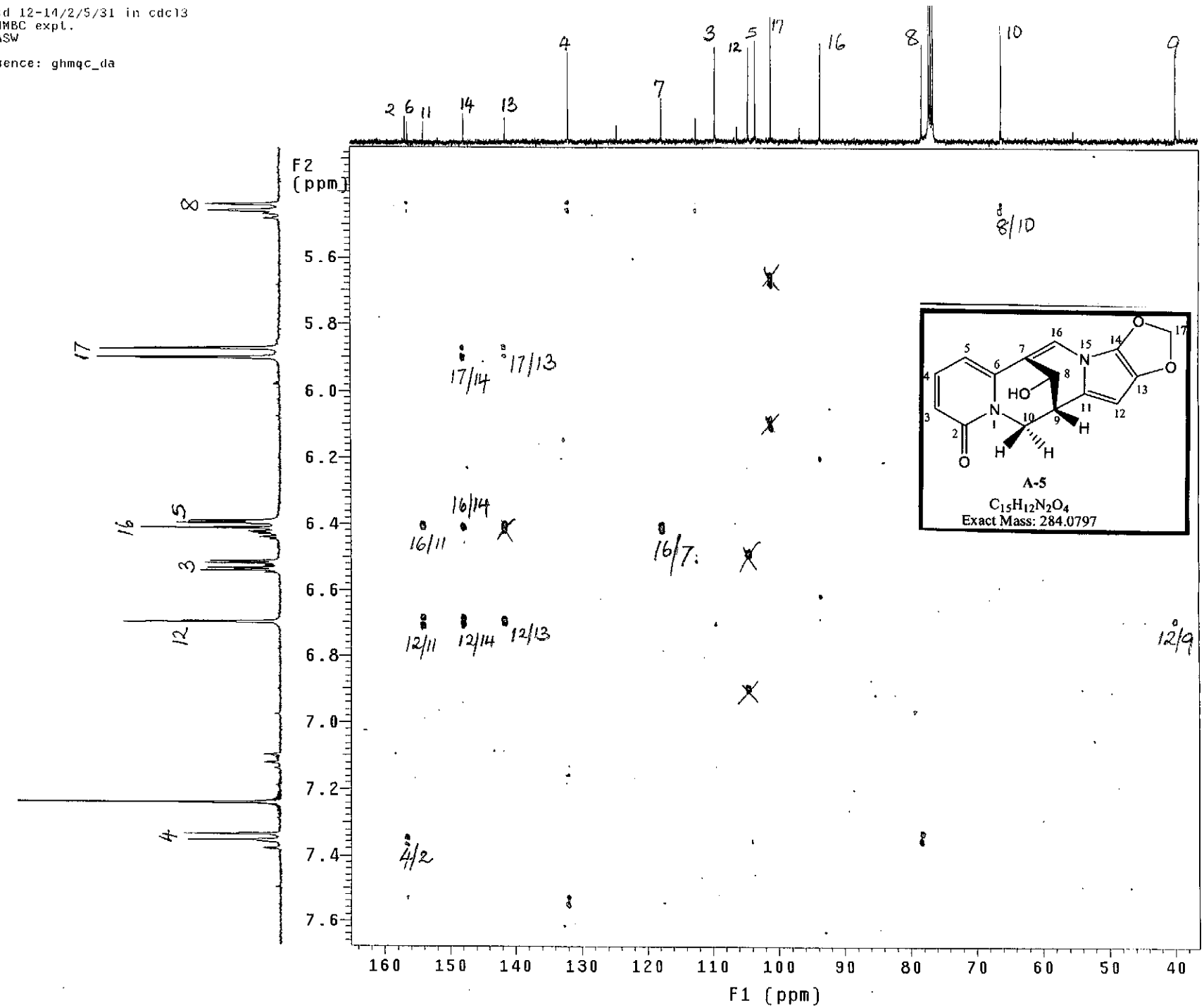
HSQC spectrum of velutinine A5

HBsv31.svsd 12-14/2/5/31 in cdcl3
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da



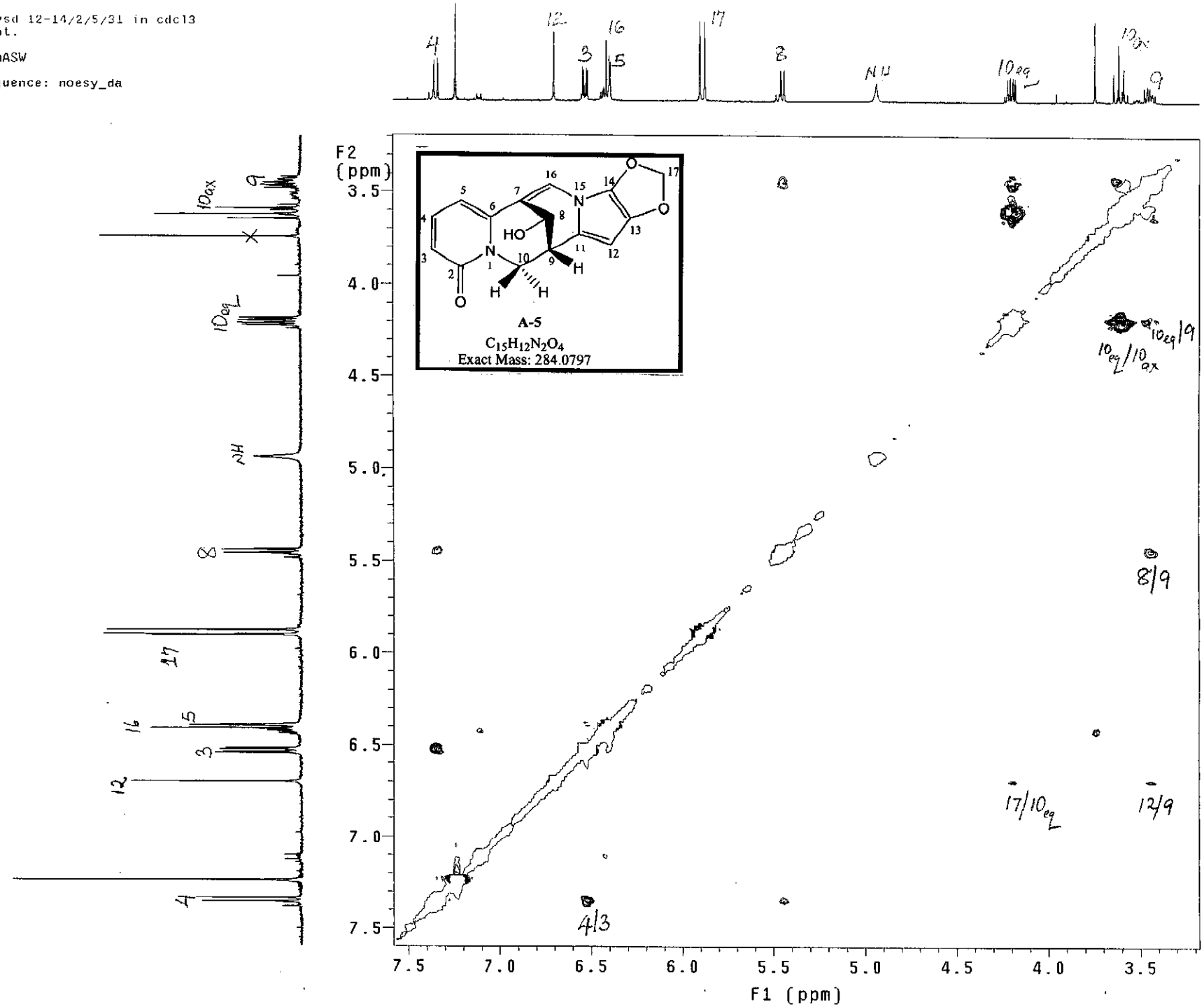
HMBC spectrum of velutinine A6



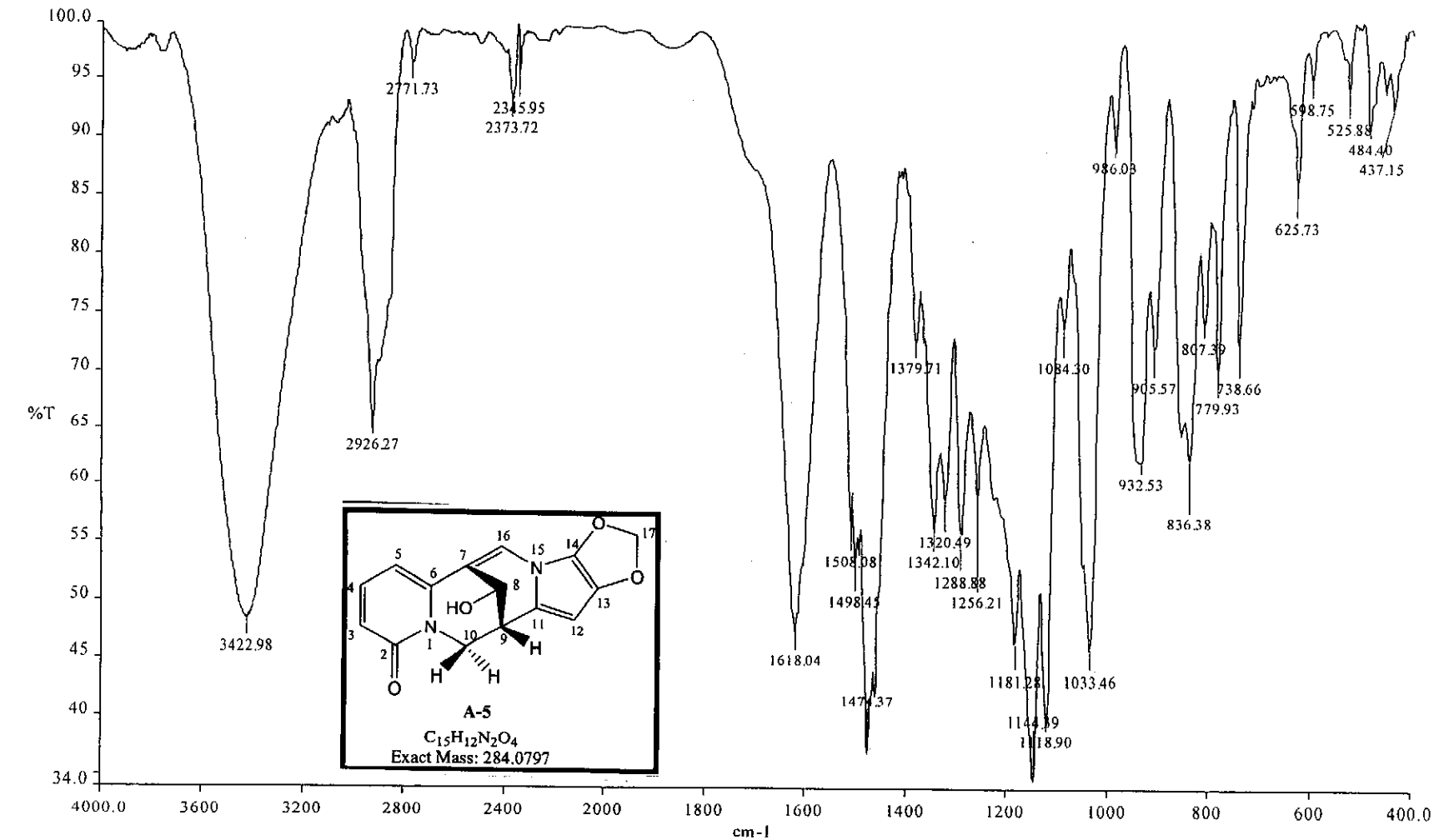
HMBC spectrum of velutinine A5

NOsv31.svsd 12-14/2/5/31 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

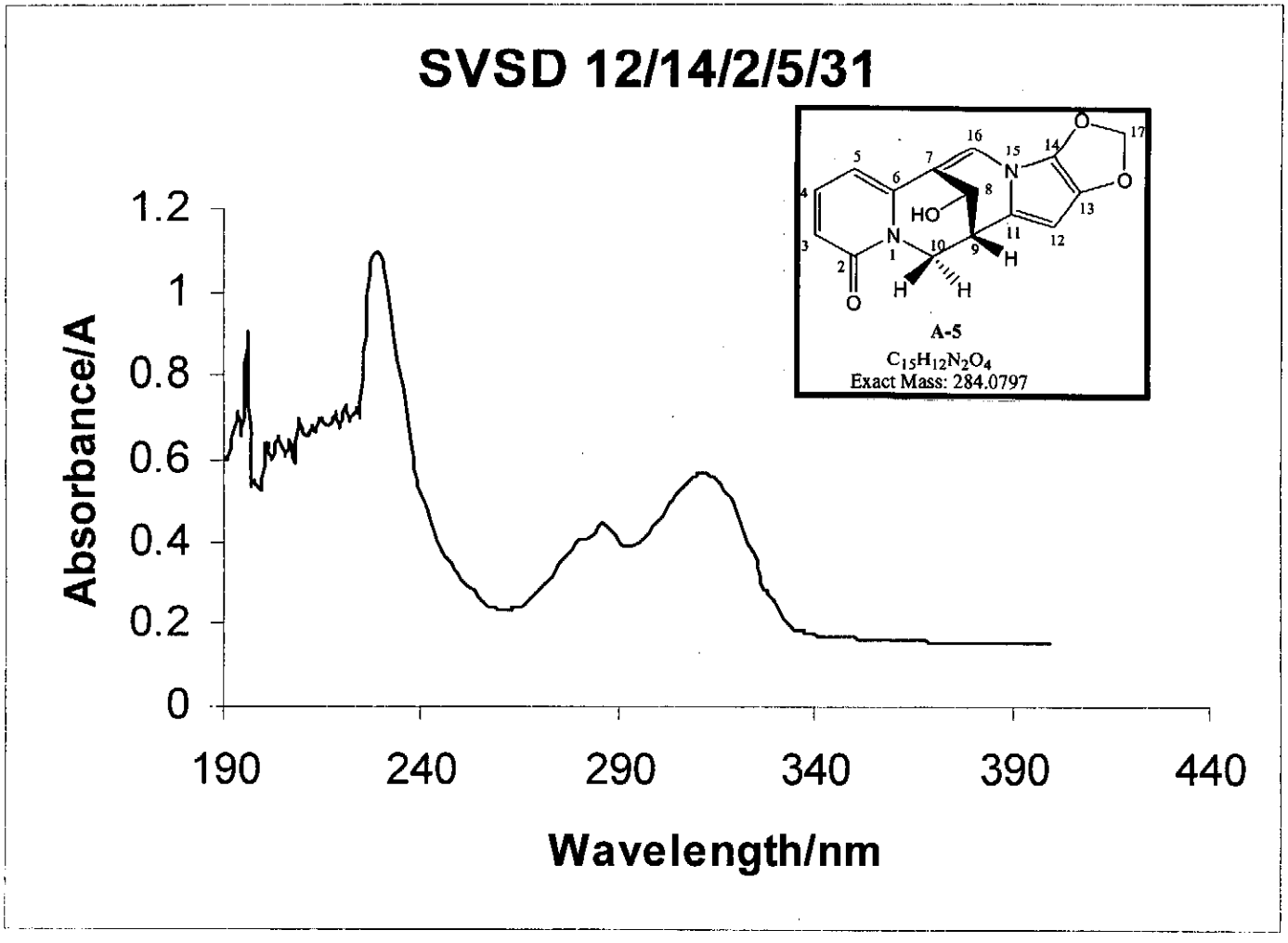


NOESY spectrum of velutinine A5

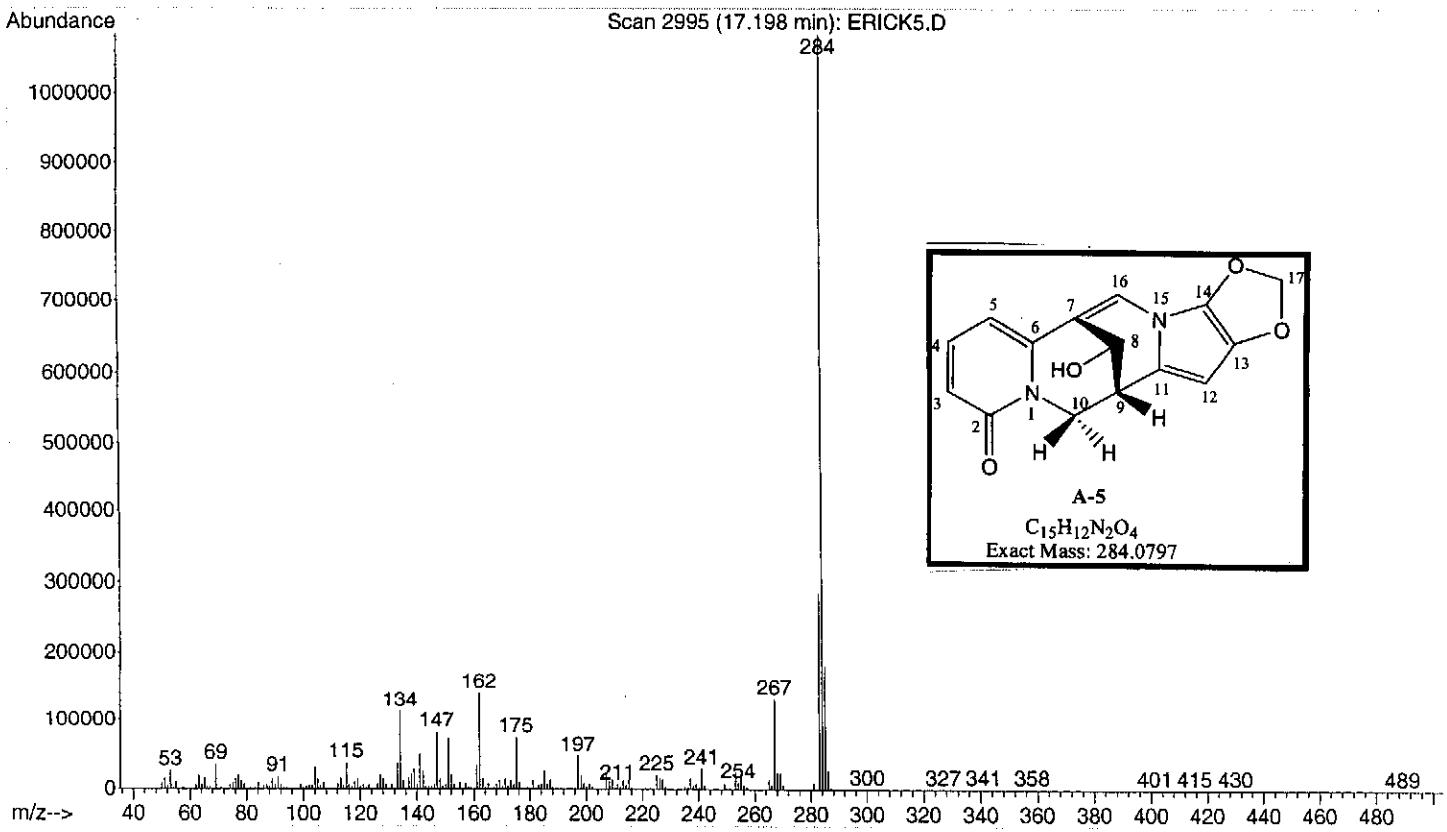


SVSD 12/14/2/5/31

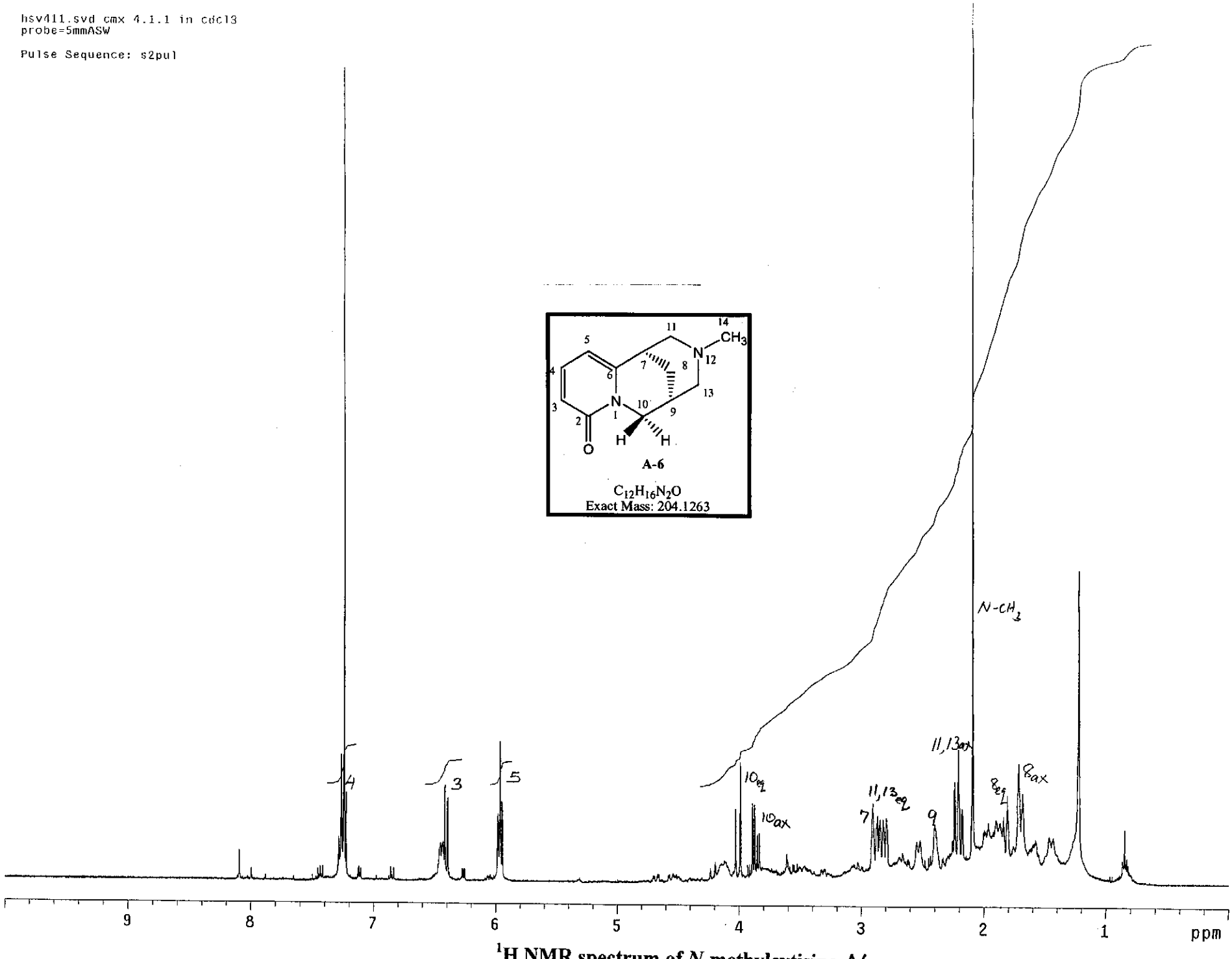
IR spectrum of velutinine A5



UV spectrum of velutinine A5



Mass spectrum of velutinine AS

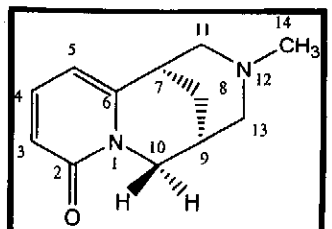


^{1}H NMR spectrum of *N*-methylcytisine A6

hsv411.svd cmx 4.1.1 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2913.133	7.284	9.0	40	958.960	2.398	12.6	79	631.341	1.579	9.4
2	2905.076	7.264	26.0	41	902.922	2.258	9.1	80	586.475	1.466	10.1
3	2900.864	7.253	8.3	42	896.147	2.241	19.8	81	573.656	1.434	9.7
4	2898.300	7.247	25.8	43	894.132	2.236	21.2	82	488.867	1.222	64.5
5	2895.553	7.240	165.4	44	885.342	2.214	19.4	83	339.616	0.849	11.5
6	2889.327	7.224	18.2	45	883.327	2.209	28.0				
7	2580.387	6.452	8.0	46	870.875	2.178	15.8				
8	2576.542	6.442	7.1	47	841.940	2.105	13.9				
9	2575.260	6.439	7.9	48	837.179	2.093	250.0				
10	2572.330	6.432	8.2	49	817.401	2.044	8.3				
11	2566.653	6.418	19.6	50	802.384	2.006	10.8				
12	2565.371	6.414	20.0	51	799.637	1.999	11.5				
13	2557.679	6.395	16.8	52	796.158	1.991	11.1				
14	2556.214	6.392	17.5	53	786.635	1.967	12.8				
15	2391.581	5.980	14.2	54	783.156	1.958	11.7				
16	2385.538	5.965	28.9	55	769.970	1.925	11.1				
17	2384.439	5.962	28.8	56	760.265	1.901	13.4				
18	2378.762	5.948	16.8	57	747.079	1.868	12.9				
19	2377.297	5.944	16.5	58	741.219	1.853	11.1				
20	1611.633	4.030	15.4	59	739.205	1.848	12.0				
21	1596.250	3.991	25.0	60	737.556	1.844	13.1				
22	1555.962	3.891	16.9	61	735.908	1.840	14.1				
23	1549.003	3.873	16.5	62	734.260	1.836	12.1				
24	1540.396	3.852	10.1	63	732.795	1.832	10.3				
25	1533.620	3.835	10.5	64	728.217	1.821	9.3				
26	1164.065	2.911	15.7	65	726.569	1.817	12.8				
27	1161.501	2.904	16.9	66	724.921	1.813	15.4				
28	1149.598	2.874	12.7	67	723.272	1.808	18.5				
29	1146.118	2.866	14.2	68	721.624	1.804	15.7				
30	1143.921	2.860	11.6	69	719.976	1.800	12.9				
31	1138.427	2.847	13.4	70	718.328	1.796	9.5				
32	1128.904	2.823	13.4	71	704.044	1.760	8.1				
33	1127.439	2.819	13.2	72	688.844	1.722	20.4				
34	1125.608	2.814	12.1	73	686.463	1.716	25.1				
35	1118.282	2.796	13.8	74	684.083	1.710	20.2				
36	1116.634	2.792	13.7	75	673.461	1.684	18.9				
37	1115.169	2.788	12.5	76	647.640	1.619	7.6				
38	1018.294	2.546	8.9	77	644.344	1.611	7.8				
39	1007.123	2.518	9.3	78	636.652	1.592	8.7				

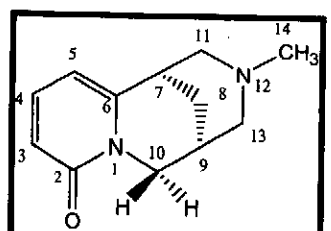


A-6

$C_{12}H_{16}N_2O$
Exact Mass: 204.1263

csv411.svd cmx 4.1.1 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul



A-6

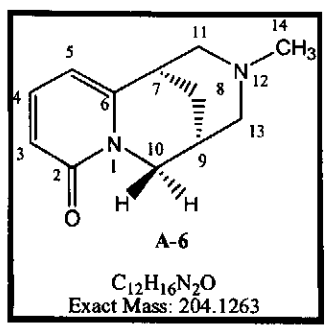
$C_{12}H_{16}N_2O$
Exact Mass: 204.1263

^{13}C NMR spectrum of *N*-methylcytisine A6

csv411.svd cmx 4.1.1 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	16459.557	163.672	4.8	40	2804.813	27.891	31.6
2	16449.639	163.573	9.9	41	2621.702	26.070	5.8
3	16271.105	161.798	6.2	42	2549.220	25.349	43.6
4	15224.320	151.389	4.5	43	2510.309	24.962	9.2
5	14001.291	139.227	6.9	44	2508.020	24.939	9.6
6	13967.721	138.893	9.2	45	2279.132	22.663	4.9
7	13940.254	138.620	29.7	46	2064.739	20.531	8.8
8	13871.587	137.937	5.1	47	2053.295	20.418	10.1
9	12707.306	126.360	5.9	48	1970.895	19.598	6.9
10	11887.884	118.211	5.8				
11	11734.529	116.687	33.2				
12	10928.077	108.667	4.8				
13	10709.107	106.490	4.9				
14	10526.759	104.677	24.9				
15	7775.515	77.319	237.1				
16	7764.071	77.205	15.4				
17	7743.471	77.000	250.0				
18	7711.426	76.681	248.4				
19	6383.871	63.480	6.3				
20	6311.390	62.760	18.3				
21	6278.582	62.433	37.3				
22	6245.775	62.107	32.9				
23	5094.464	50.659	7.0				
24	5021.220	49.930	36.0				
25	4922.797	48.952	5.1				
26	4641.264	46.152	35.5				
27	4457.390	44.324	4.6				
28	4407.798	43.831	6.4				
29	4338.368	43.140	5.7				
30	4322.346	42.981	6.7				
31	3946.205	39.241	5.2				
32	3557.857	35.379	27.0				
33	3333.546	33.148	7.2				
34	3254.198	32.359	18.2				
35	3225.205	32.071	5.9				
36	3206.894	31.889	4.6				
37	3054.302	30.372	5.7				
38	2983.346	29.666	16.6				
39	2946.724	29.302	4.9				



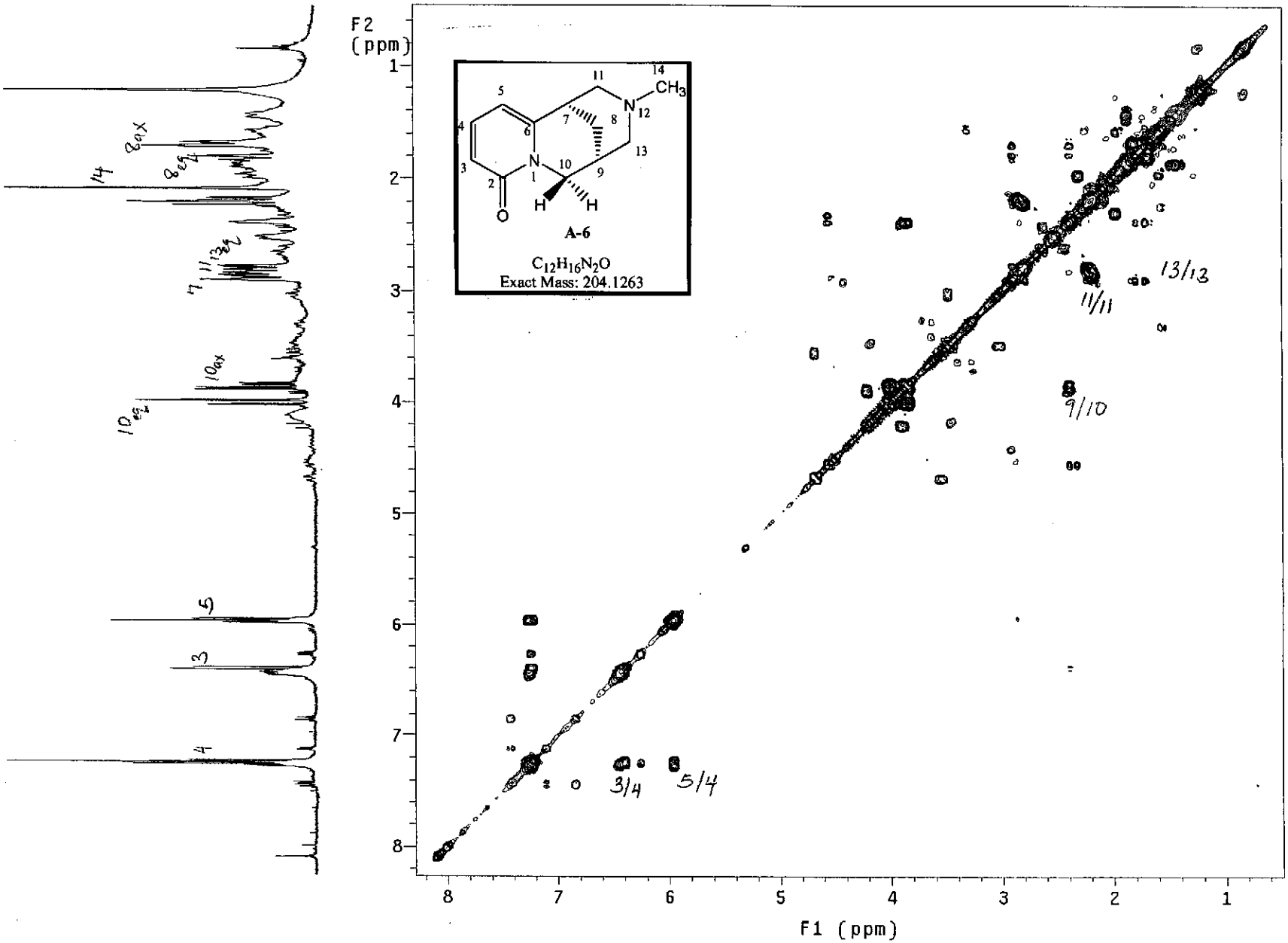
A-6

$C_{12}H_{16}N_2O$

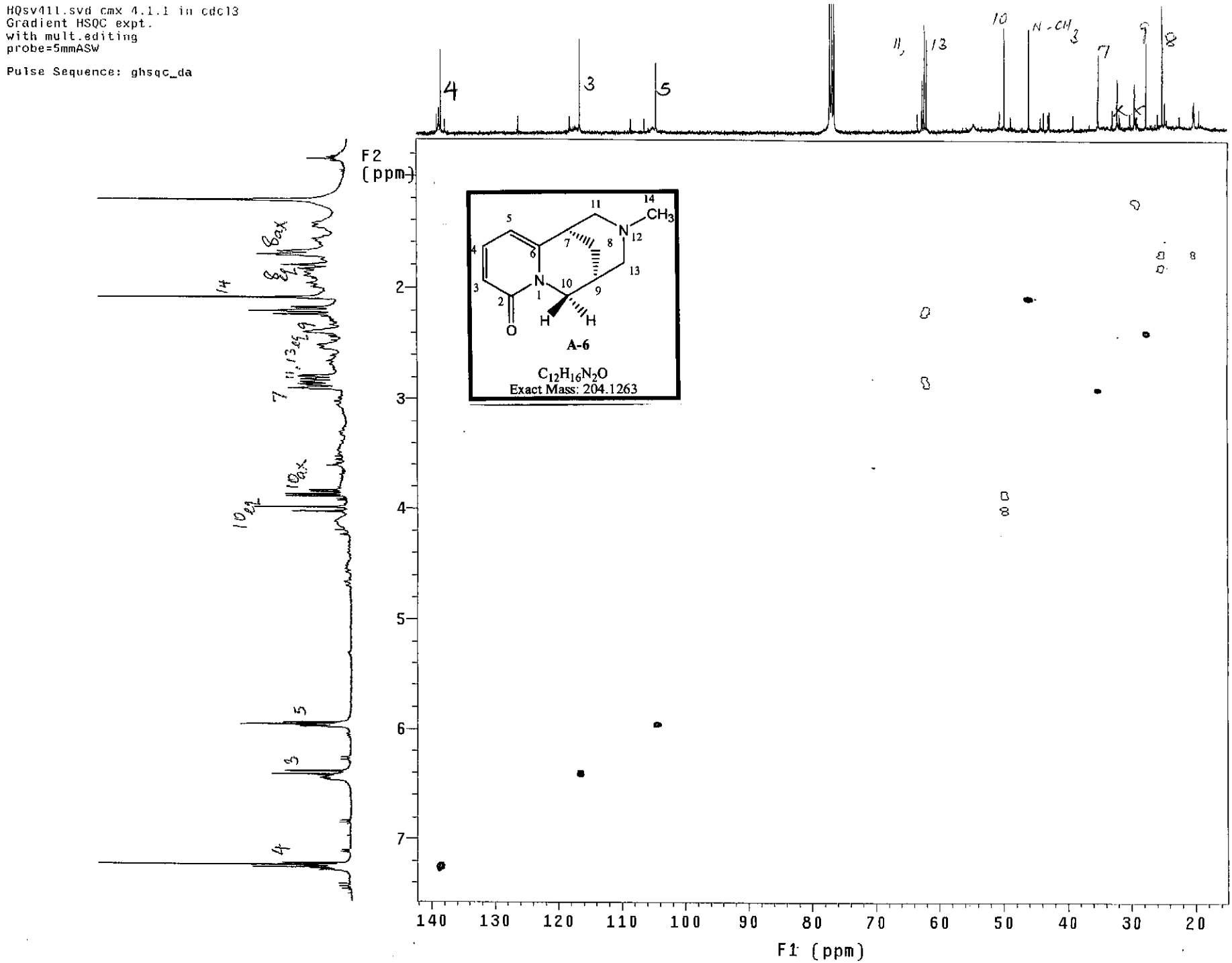
Exact Mass: 204.1263

cysv411.svd cmx 4.1.1 in cdcl3
1H Cosy-90
probe=5mmASW

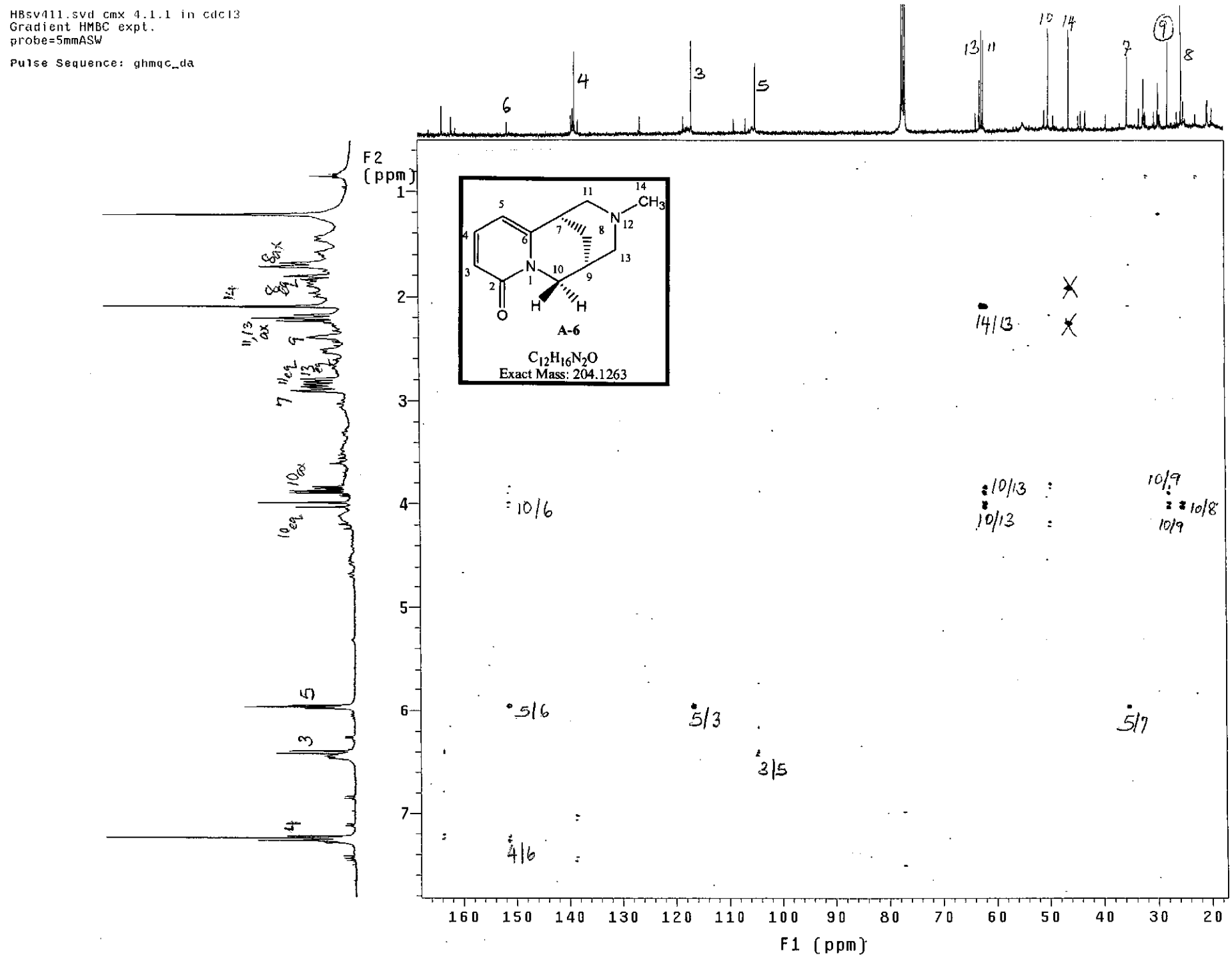
Pulse Sequence: relayh



COSY spectrum of *N*-methylcytisine A6



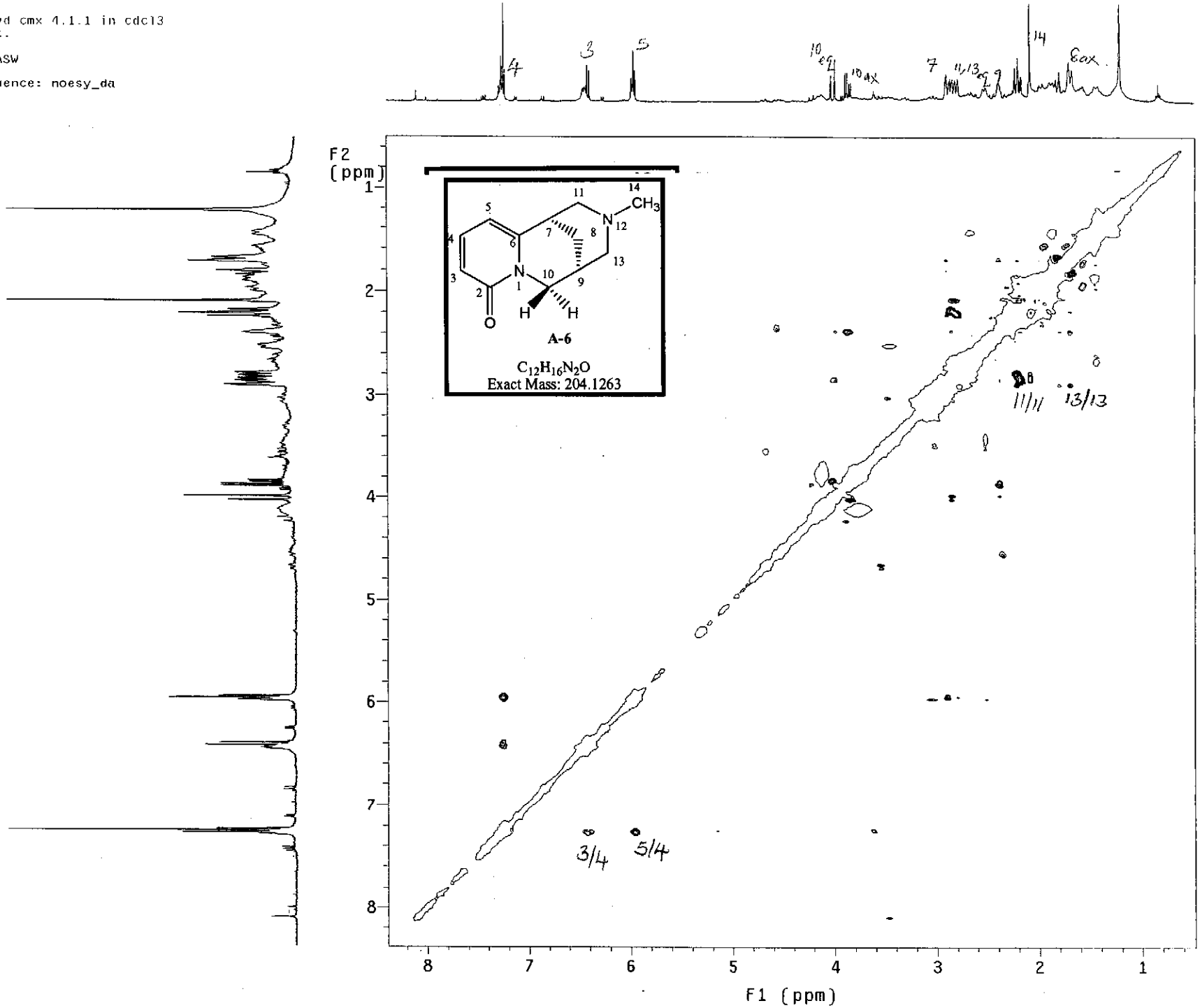
HSQC spectrum of *N*-methylcytisine A6



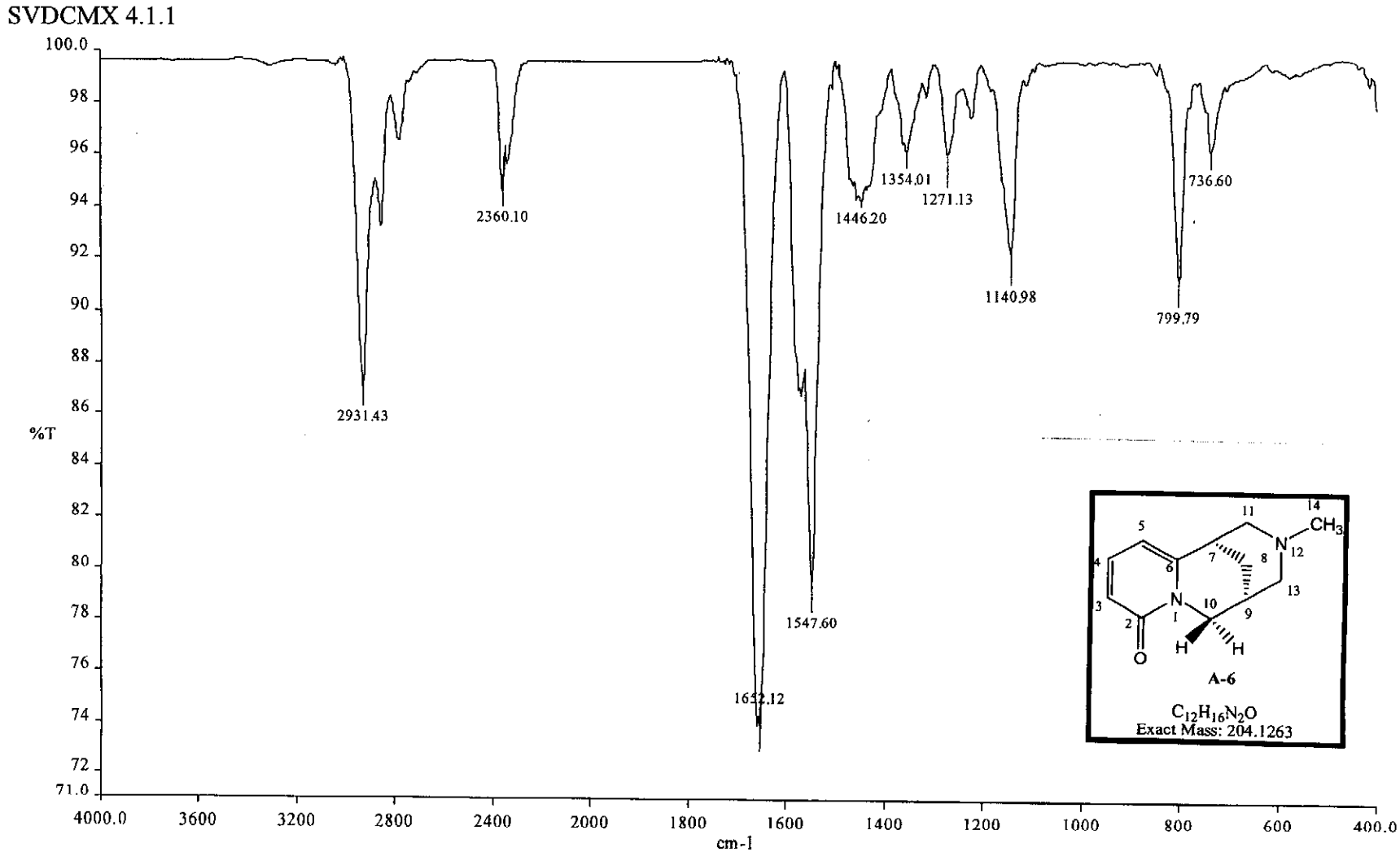
HMBC spectrum of *N*-methylcytisine A6

NOsv411.svd cmx 4.1.1 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW

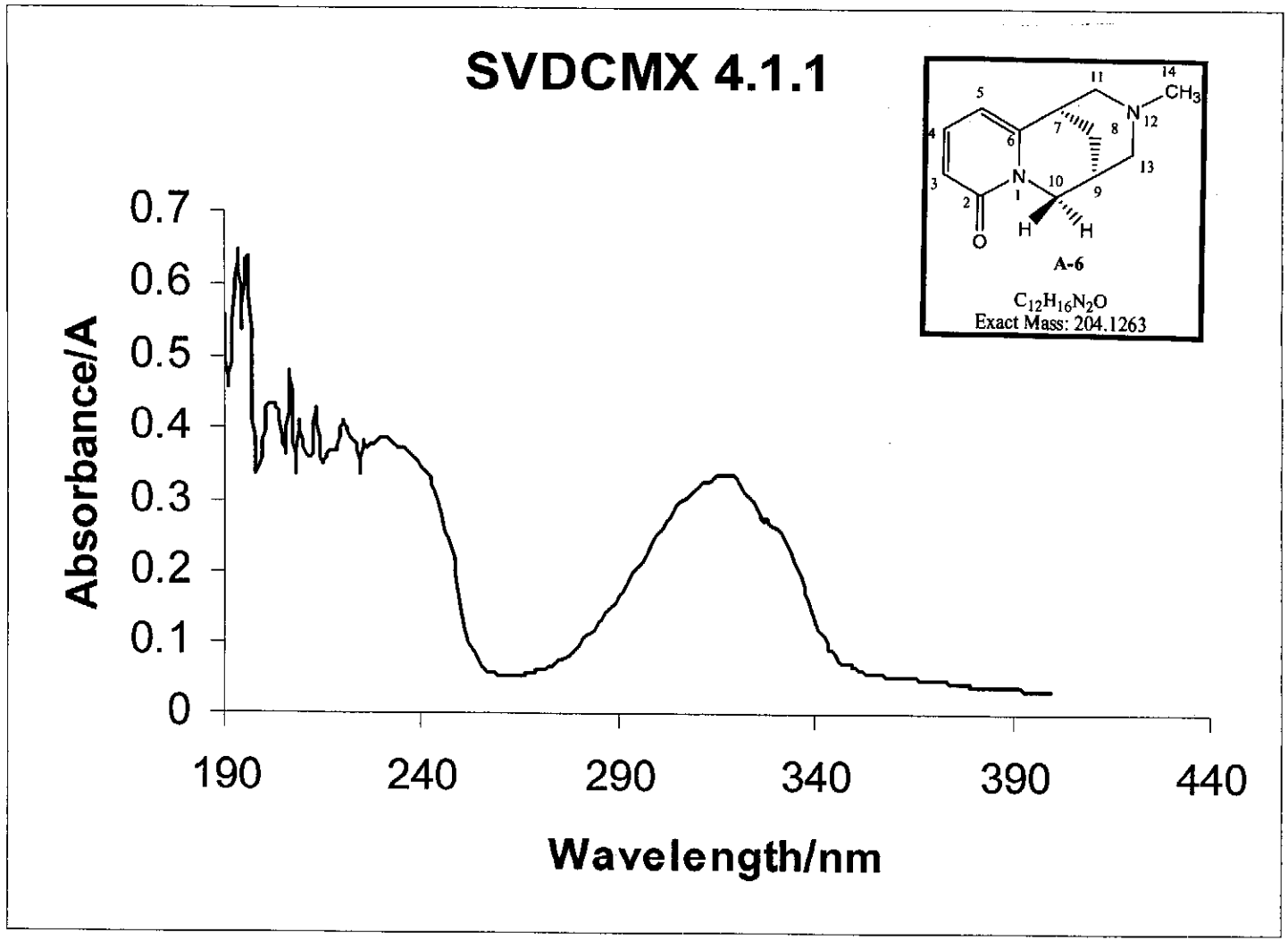
Pulse Sequence: noesy_da



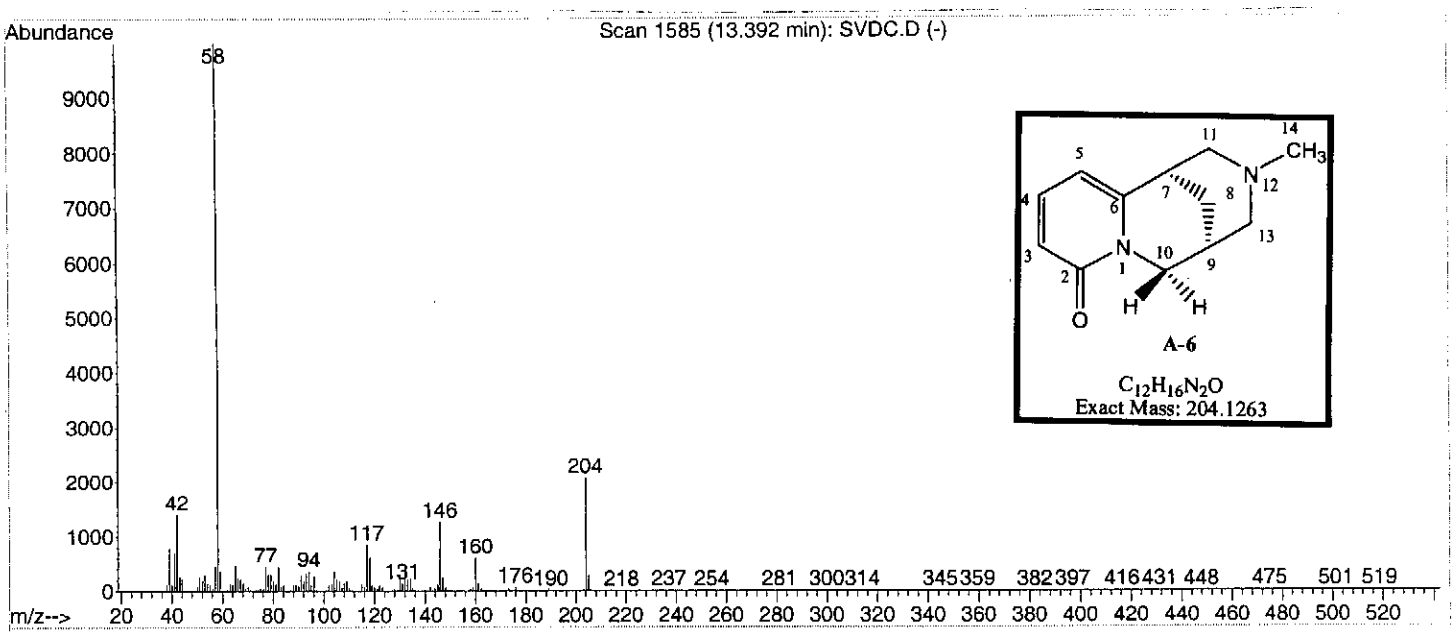
NOESY spectrum of *N*-methylcytisine A6



IR spectrum of N-methylcytisine A6



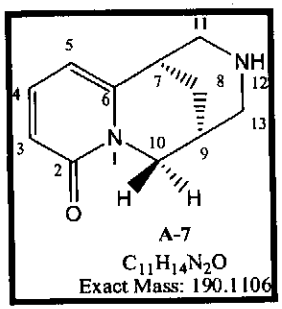
UV spectrum of *N*-methylcytisine A6



Mass spectrum of *N*-methylcytisine A6

hsv12c.svmld 30-33/12C in cdcl3
probe=5mmASW

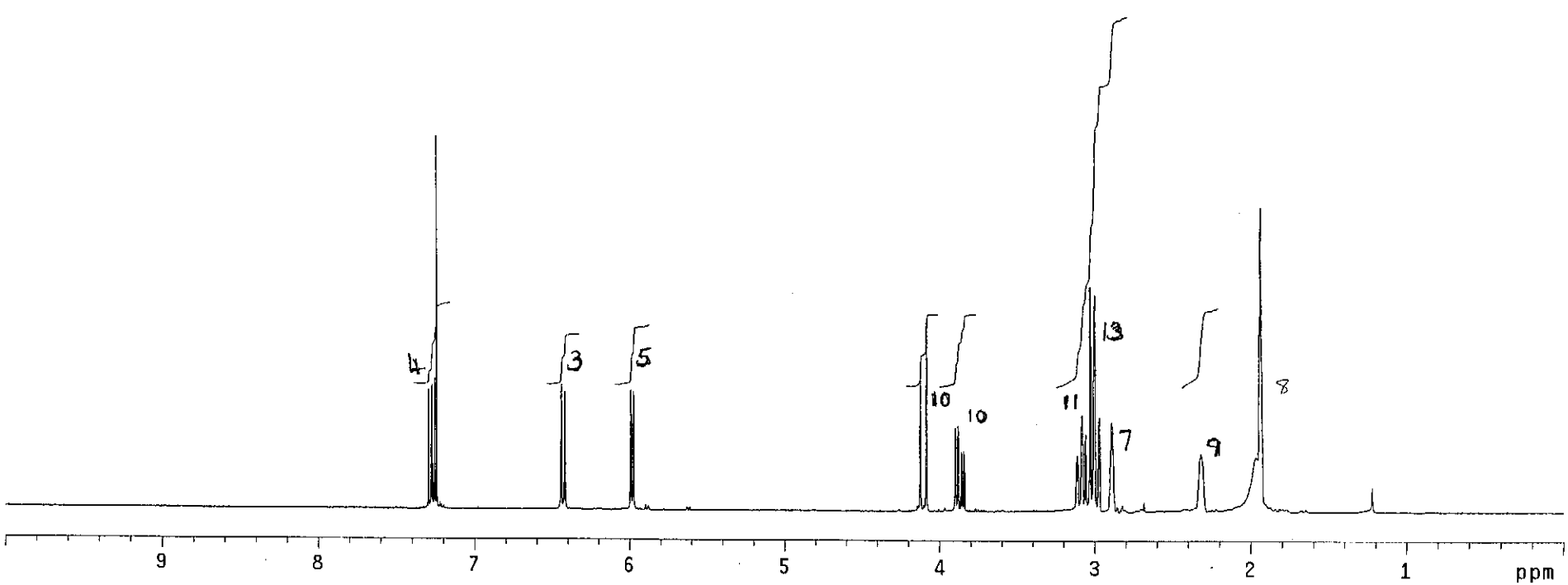
Pulse Sequence: s2pul



A-7

$C_{11}H_{14}N_2O$

Exact Mass: 190.1106

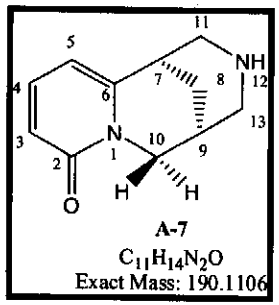


^{1}H NMR of cytisine A7

hsv12c.svmld 30-33/12C in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2915.880	7.291	19.0	40	776.929	1.943	31.6
2	2908.921	7.273	19.5	41	773.816	1.935	49.1
3	2906.907	7.268	19.8	42	770.703	1.927	25.0
4	2899.948	7.251	20.8				
5	2895.553	7.240	60.0				
6	2575.443	6.440	19.3				
7	2573.978	6.436	20.0				
8	2566.286	6.417	18.6				
9	2565.004	6.414	18.7				
10	2395.244	5.989	19.0				
11	2393.962	5.986	19.0				
12	2388.285	5.972	18.8				
13	2387.003	5.968	18.1				
14	1648.259	4.121	22.1				
15	1632.693	4.082	30.6				
16	1559.075	3.898	13.3				
17	1557.976	3.896	13.1				
18	1552.299	3.881	13.8				
19	1551.200	3.879	12.9				
20	1543.326	3.859	9.6				
21	1542.227	3.856	9.3				
22	1536.733	3.842	9.7				
23	1535.634	3.840	9.1				
24	1244.092	3.111	9.2				
25	1231.640	3.080	15.6				
26	1224.314	3.061	11.6				
27	1221.934	3.055	12.4				
28	1212.228	3.031	36.1				
29	1209.847	3.025	36.2				
30	1201.973	3.005	32.4				
31	1200.691	3.002	33.3				
32	1199.592	2.999	35.0				
33	1189.520	2.974	13.9				
34	1188.238	2.971	14.9				
35	1187.139	2.968	15.1				
36	1156.190	2.891	14.5				
37	1154.176	2.886	13.9				
38	926.912	2.318	9.5				
39	786.269	1.966	9.1				



A-7

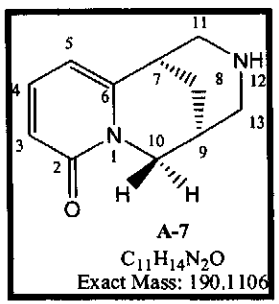
$C_{11}H_{14}N_2O$

Exact Mass: 190.1106

csv12c.svmld 30-33/12C in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

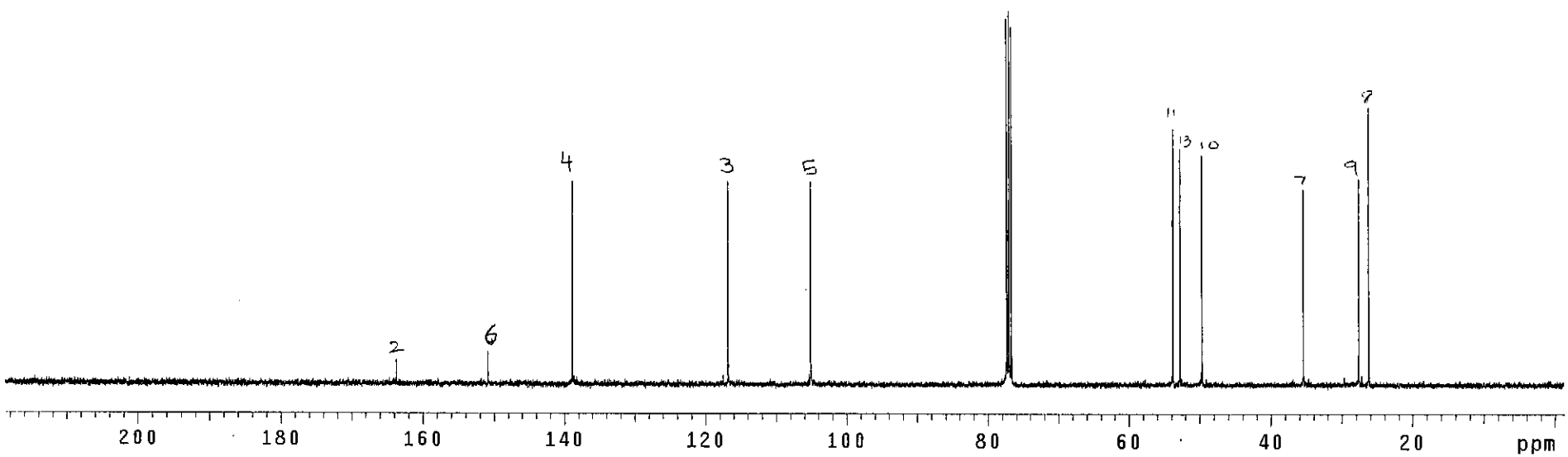
INDEX	FREQUENCY	PPM	HEIGHT
1	16458.794	163.664	3.8
2	15167.098	150.820	5.2
3	13960.854	138.825	32.7
4	11745.973	116.800	32.7
5	10568.722	105.094	32.7
6	7775.515	77.319	58.7
7	7743.471	77.000	60.0
8	7711.426	76.681	57.4
9	5403.464	53.731	41.3
10	5303.516	52.737	38.0
11	4993.753	49.657	36.9
12	3560.909	35.409	31.7
13	2776.583	27.610	33.4
14	2631.620	26.168	44.7



A-7

$C_{11}H_{14}N_2O$

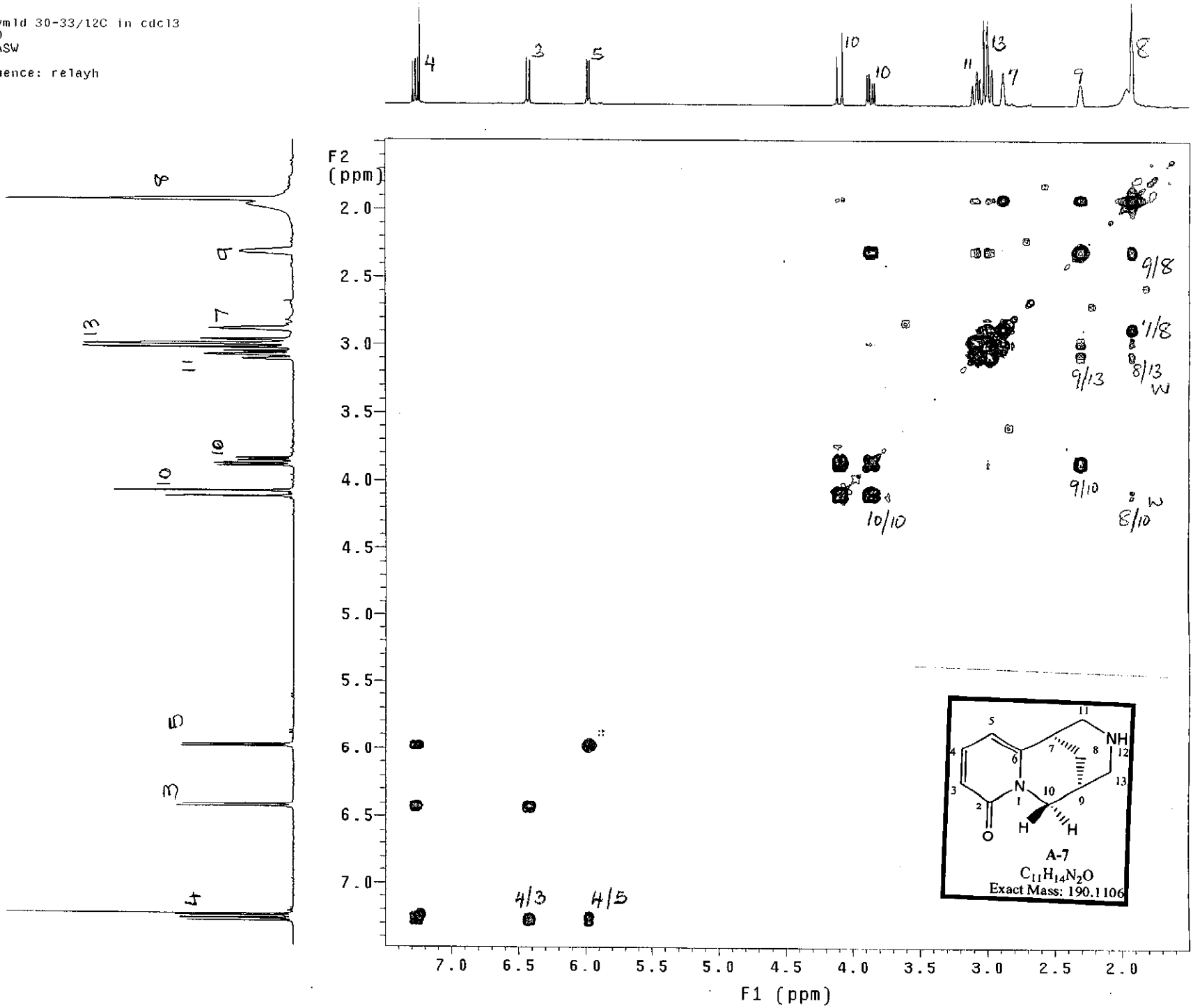
Exact Mass: 190.1106



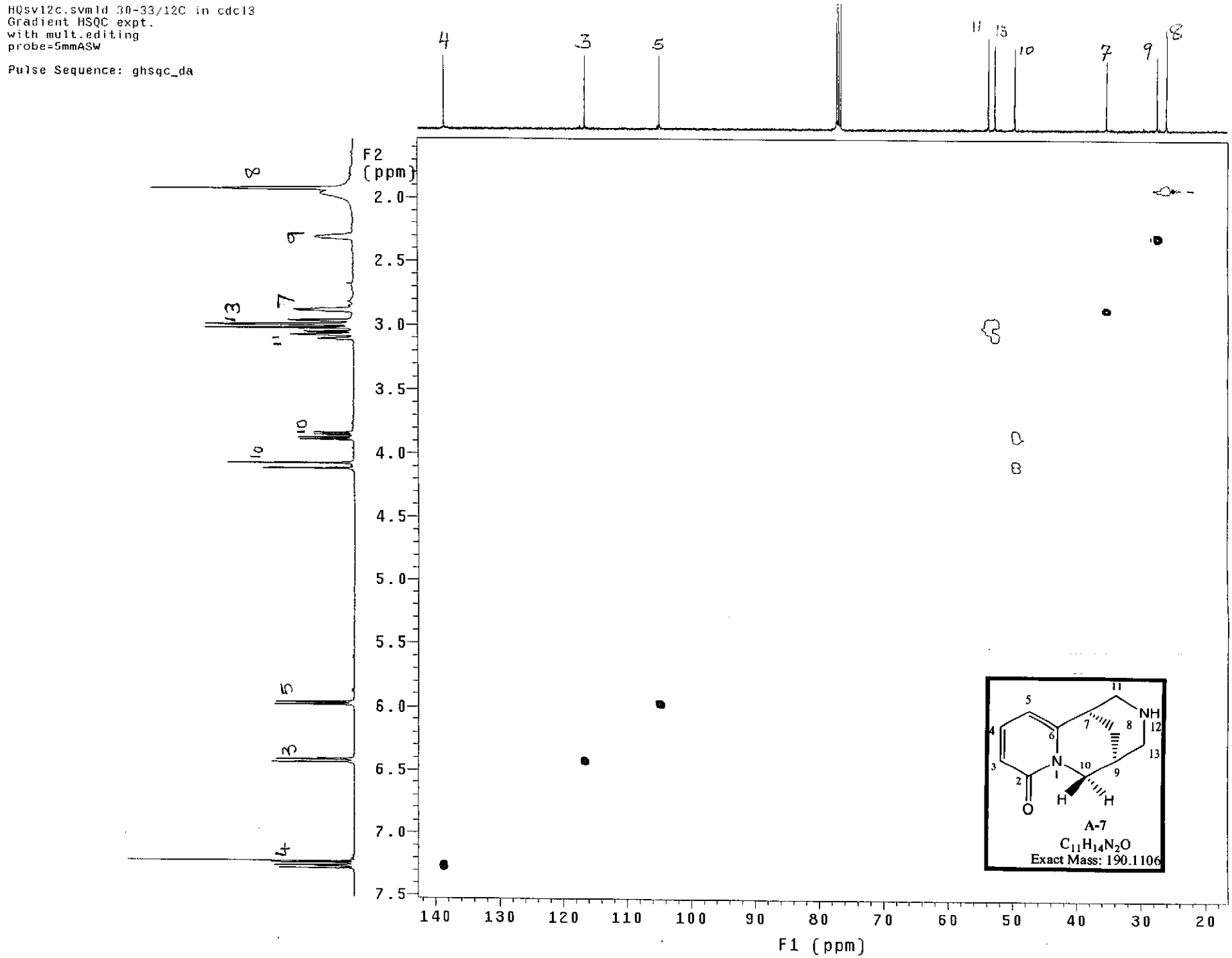
^{13}C NMR spectrum of cytisine A-7

cysv12c.svmld 30-33/12C in cdcl3
1H Cosy-90
probe=5mmASW

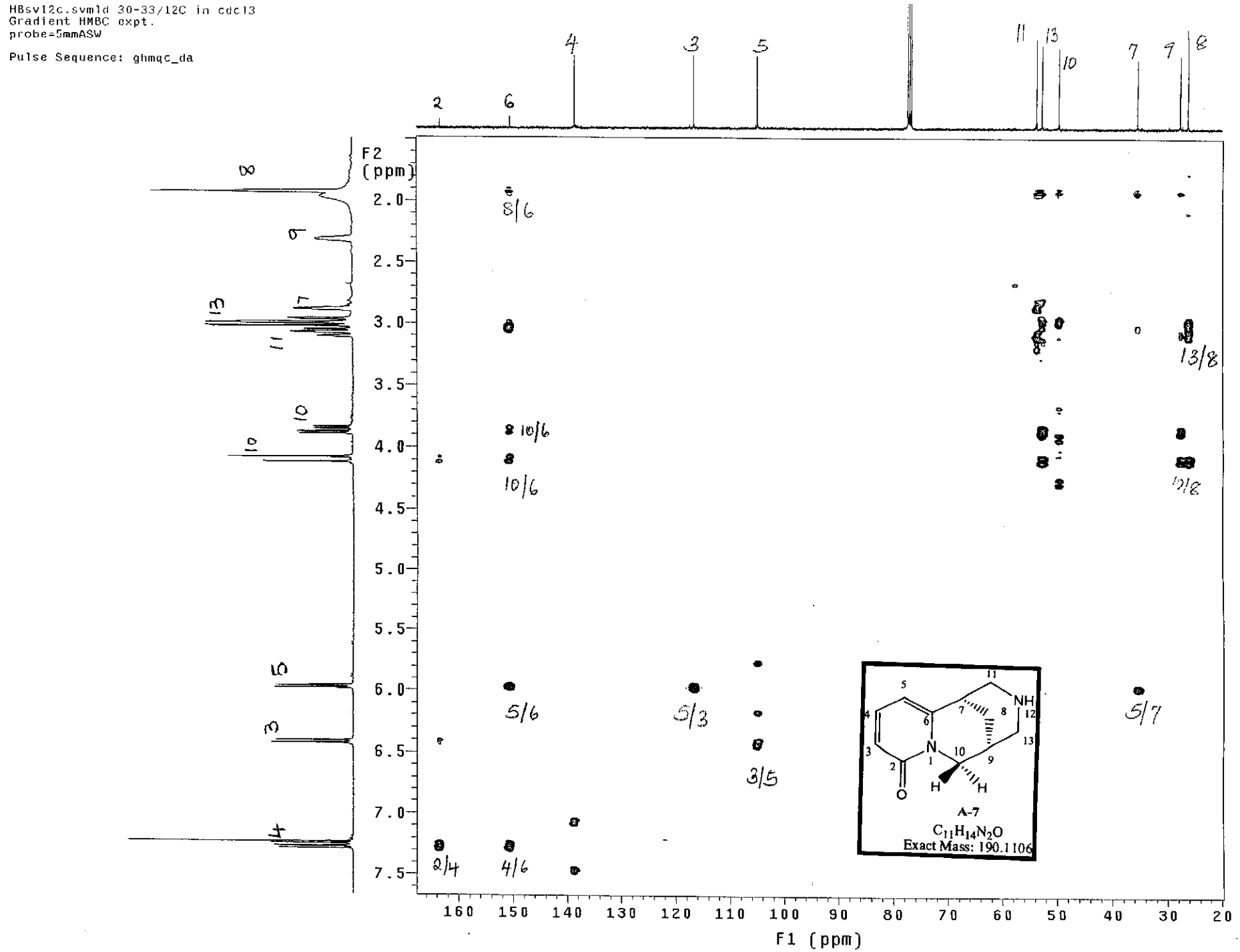
Pulse Sequence: relayh



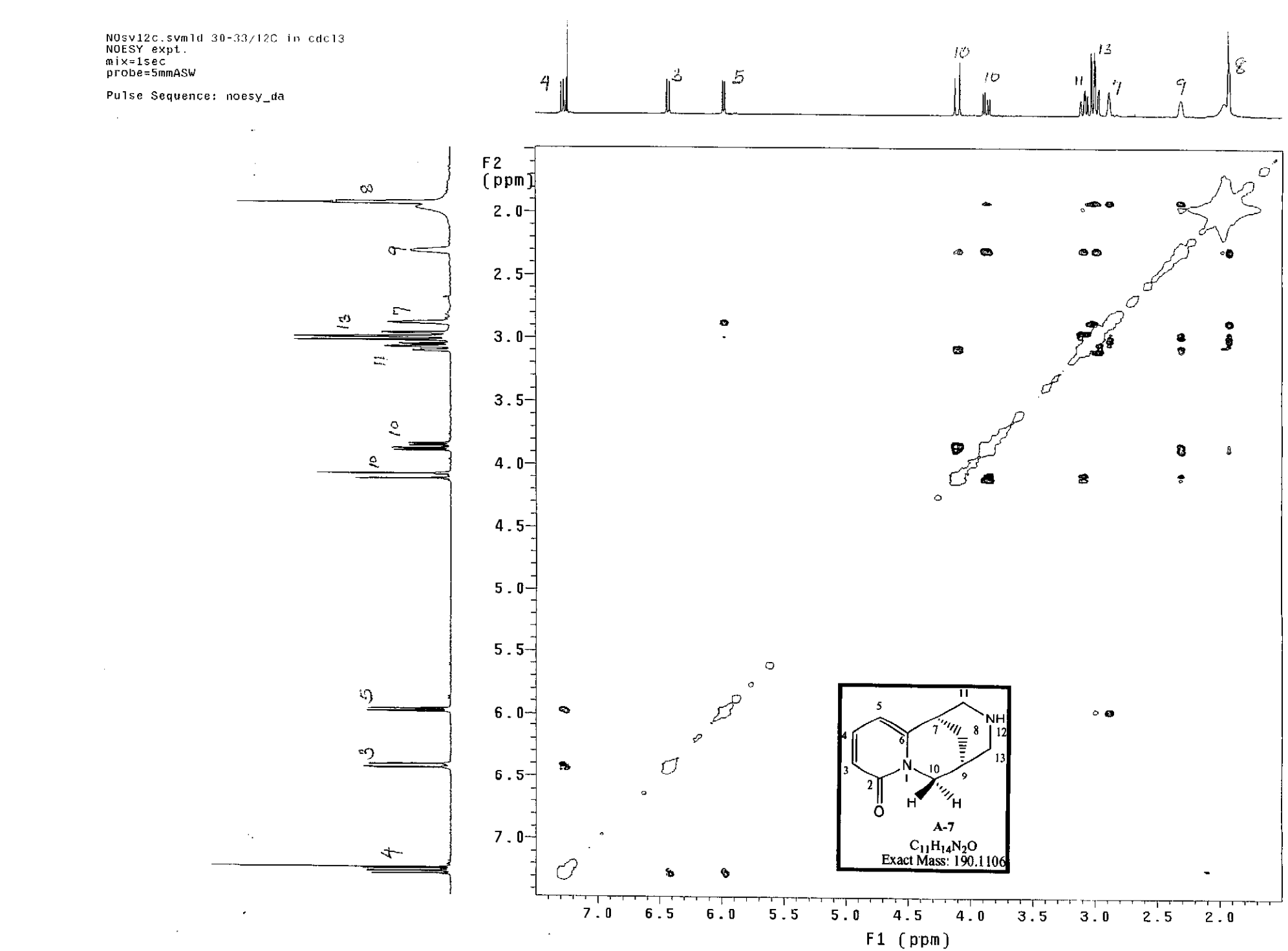
COSYspectrum of cytisine A7



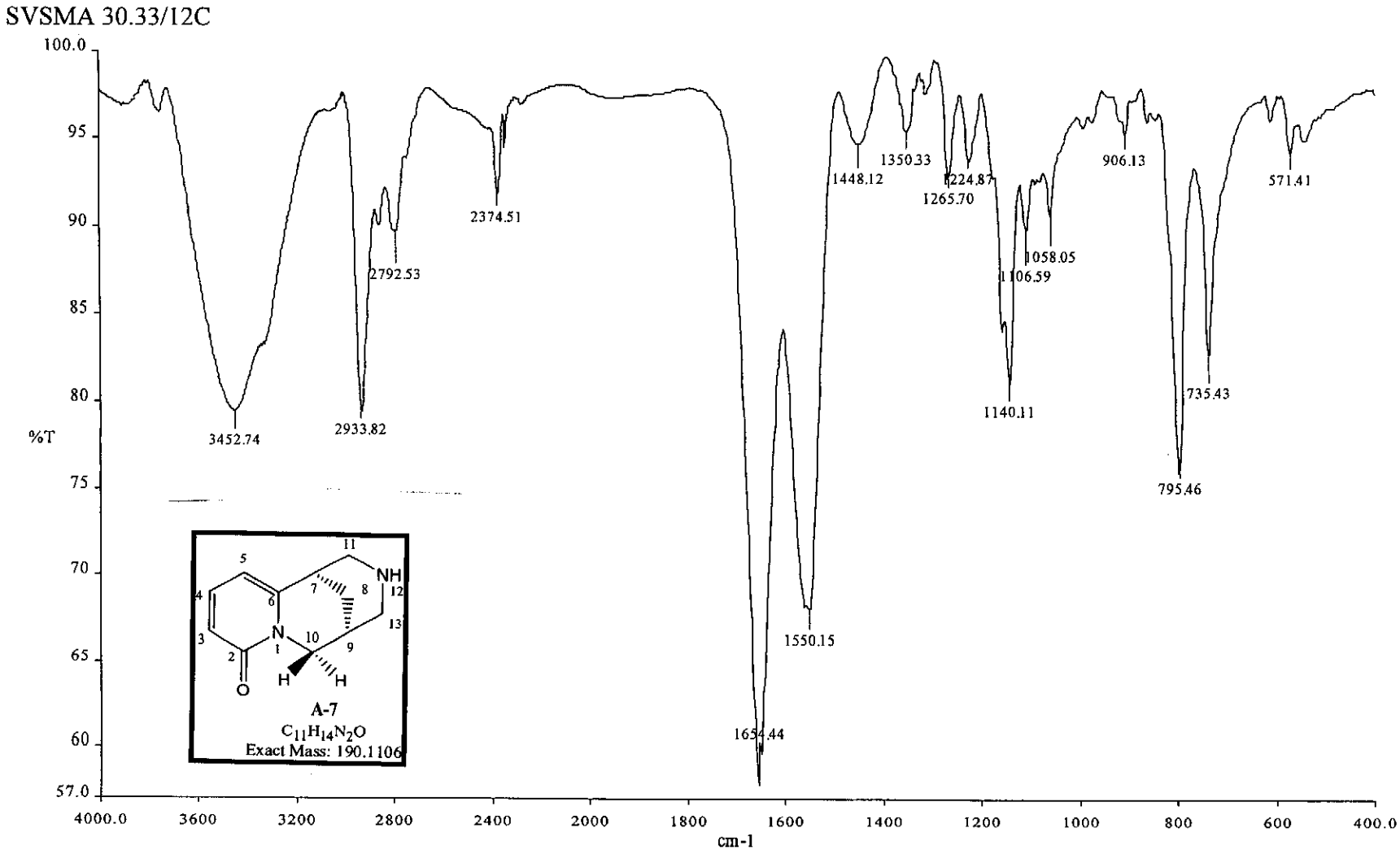
HSQC spectrum of cytisine A-7



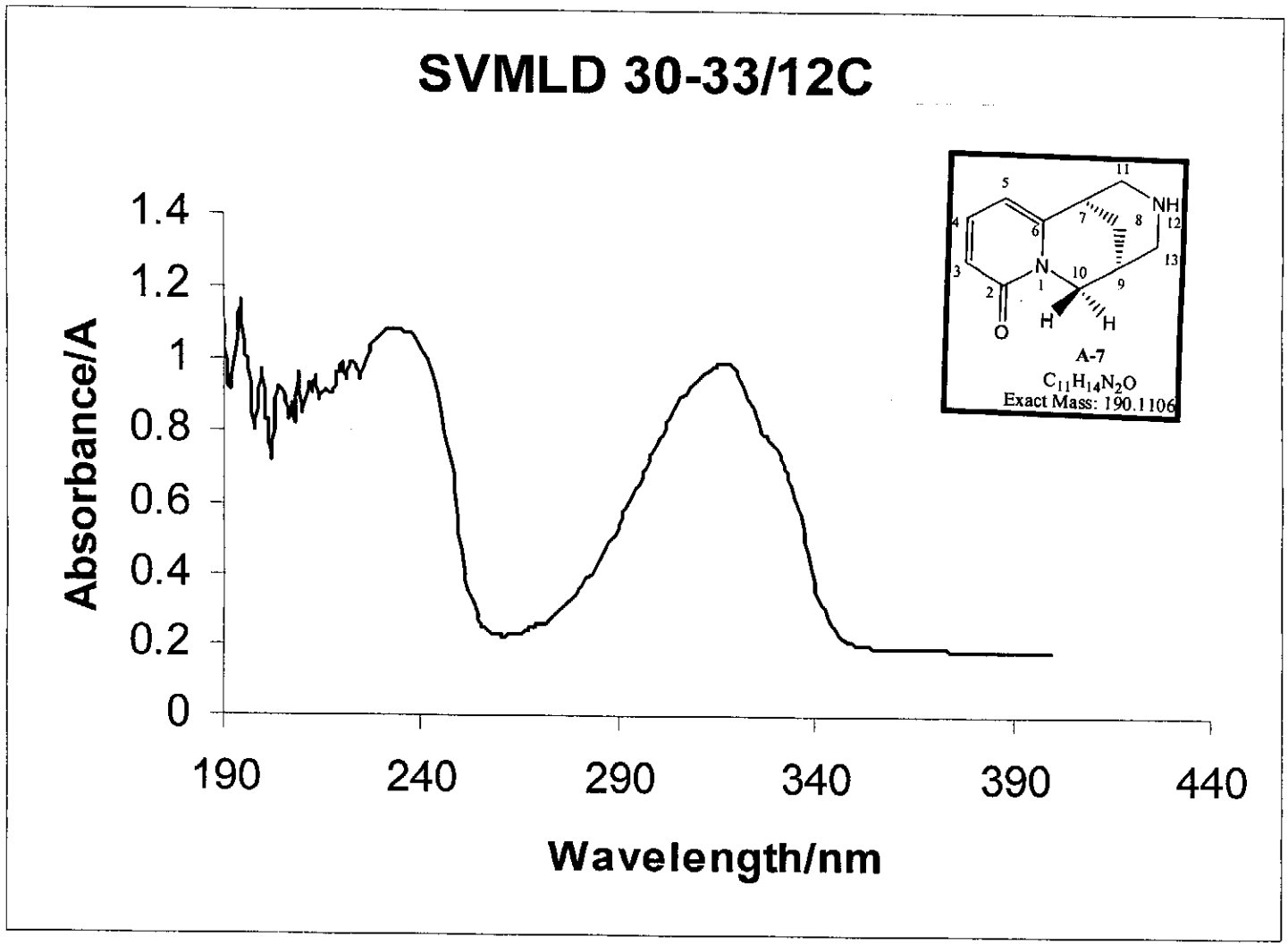
HMBC spectrum of cytisine A7



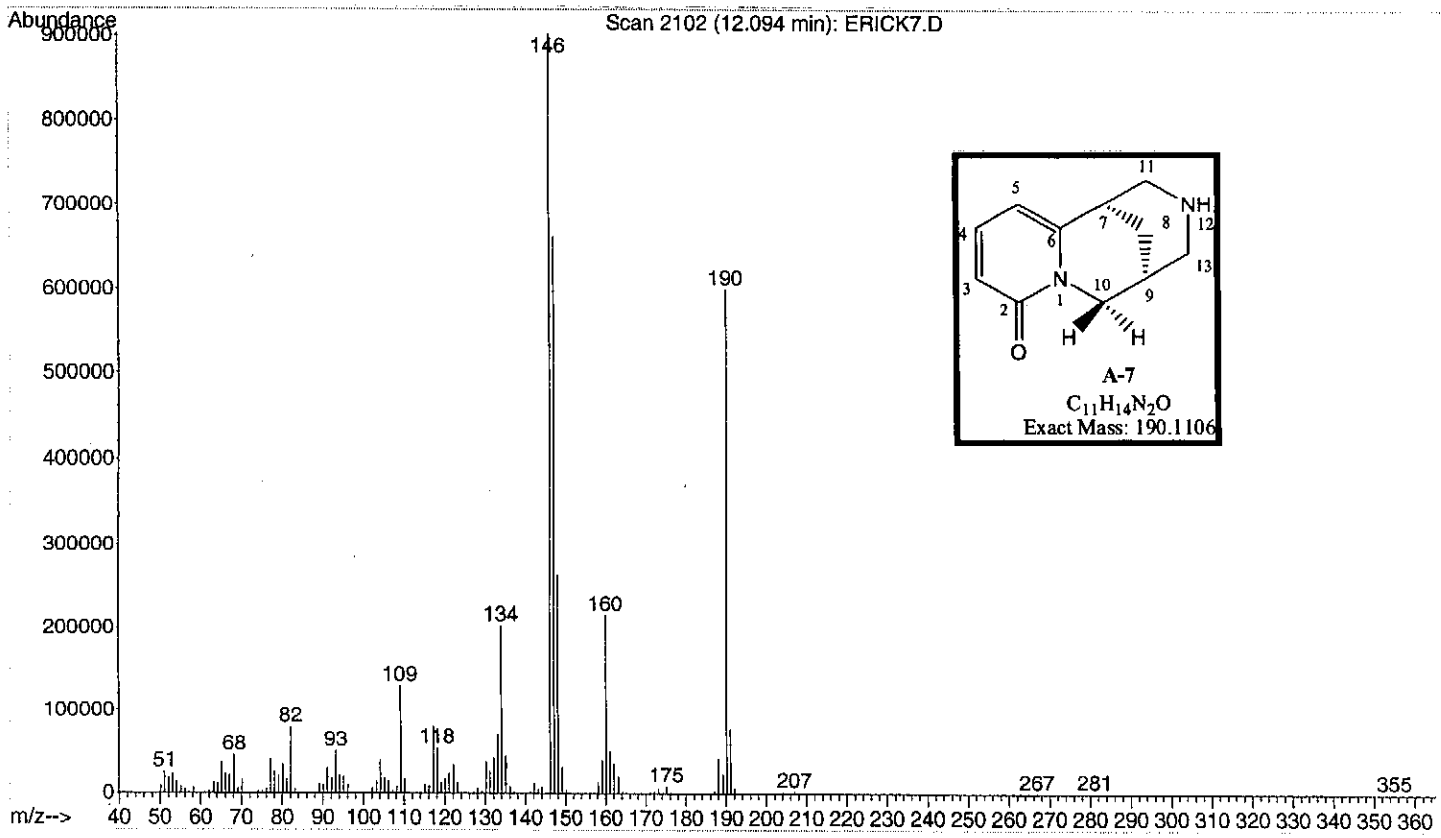
NOESY spectrum of cytisine A7



IR spectrum of cytisine A7



UV spectrum of cytisine A7

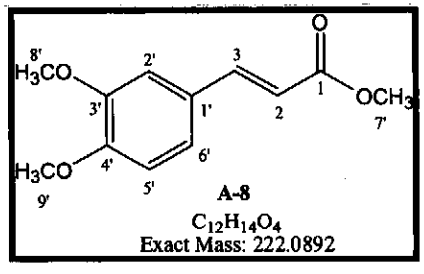


Mass spectrum of cytisine A7

probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	3055.242	7.639	7.8
2	3039.310	7.599	8.2
3	2895.553	7.240	68.1
4	2839.332	7.099	4.4
5	2837.684	7.095	4.9
6	2831.091	7.079	5.1
7	2829.077	7.074	5.9
8	2812.229	7.032	11.0
9	2810.215	7.027	8.9
10	2743.006	6.859	11.4
11	2734.582	6.838	9.9
12	2525.265	6.314	14.4
13	2509.333	6.274	13.5
14	1563.104	3.908	4.8
15	1559.807	3.900	23.6
16	1557.427	3.894	150.0
17	1555.228	3.889	24.6
18	1511.827	3.780	95.1
19	1509.813	3.775	6.8
20	1506.517	3.767	7.3
21	1481.317	3.729	7.7
22	1485.823	3.715	22.5
23	1441.506	3.604	6.3
24	623.284	1.558	14.5
25	491.431	1.229	24.5



A-8

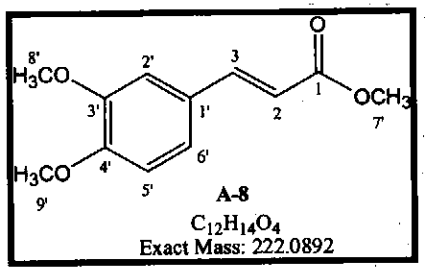
$C_{12}H_{14}O_4$

Exact Mass: 222.0892

^{1}H NMR spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8

csvo.svsu 12-14/1/6 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul



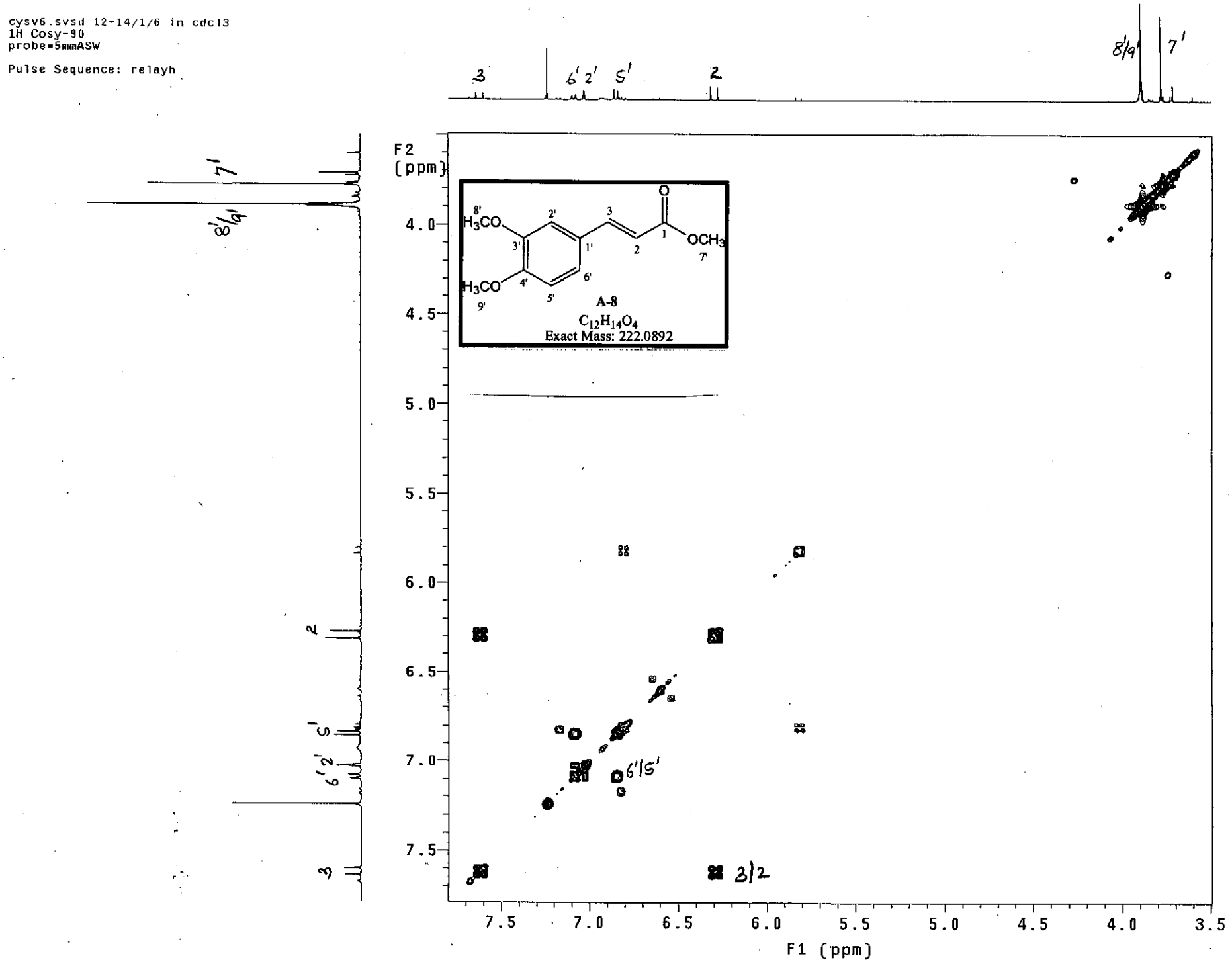
A-8

$C_{12}H_{14}O_4$

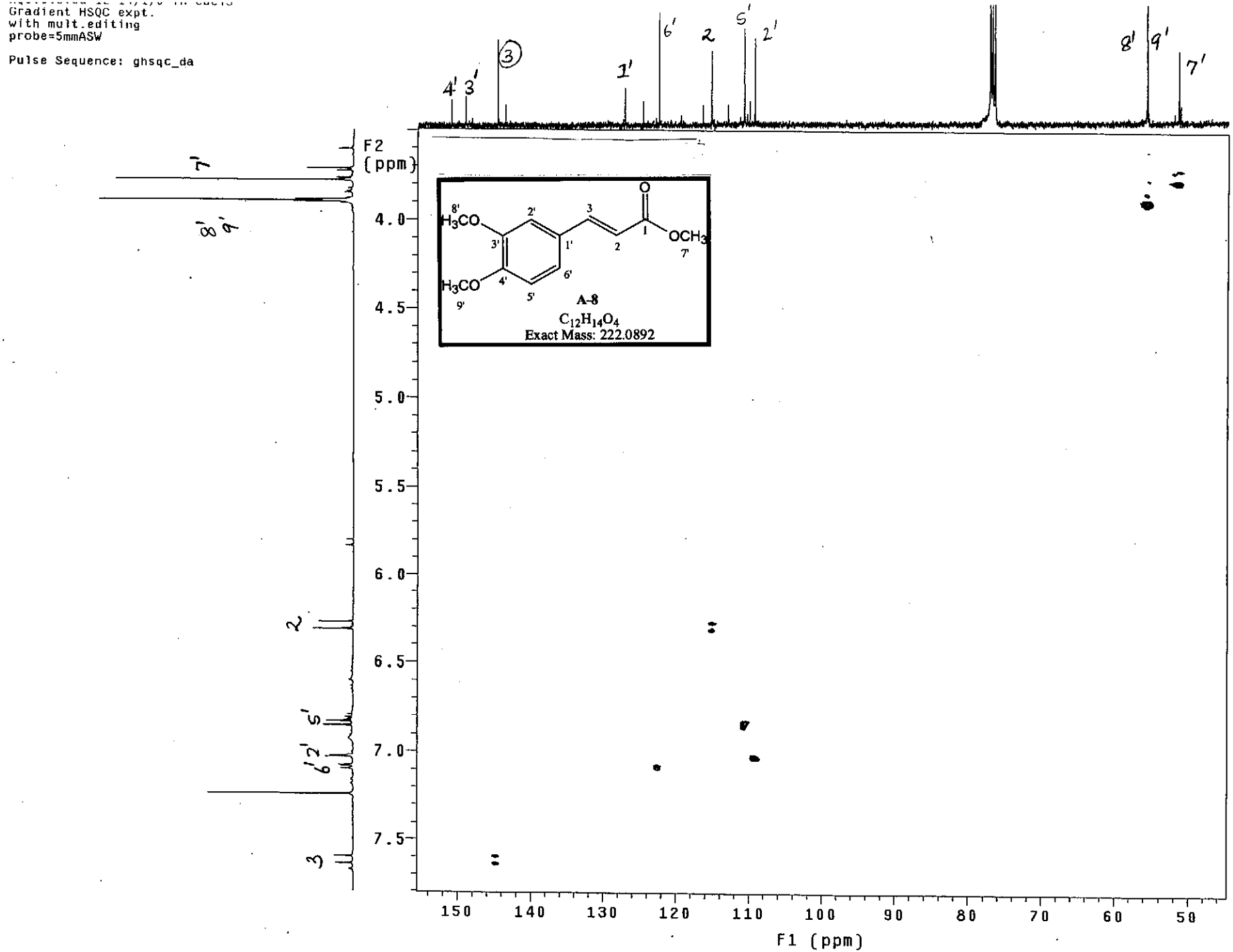
Exact Mass: 222.0892

INDEX	FREQUENCY	PPM	HEIGHT
1	16863.164	167.685	7.4
2	15195.328	151.100	6.0
3	15001.535	149.173	6.9
4	14560.543	144.788	20.2
5	14457.543	143.764	4.8
6	12804.965	127.331	8.7
7	12549.373	124.789	5.5
8	12329.640	122.604	26.1
9	11730.714	116.649	4.6
10	11610.929	115.457	17.4
11	11379.751	113.159	4.7
12	11160.781	110.981	22.5
13	11087.536	110.253	5.6
14	11016.581	109.547	20.3
15	7775.515	77.319	197.4
16	7764.071	77.205	12.9
17	7743.471	77.000	200.0
18	7711.426	76.681	192.5
19	5627.775	55.962	25.8
20	5617.858	55.863	29.3
21	5192.123	51.630	17.0
22	5167.708	51.387	4.0
23	2985.635	29.689	15.8

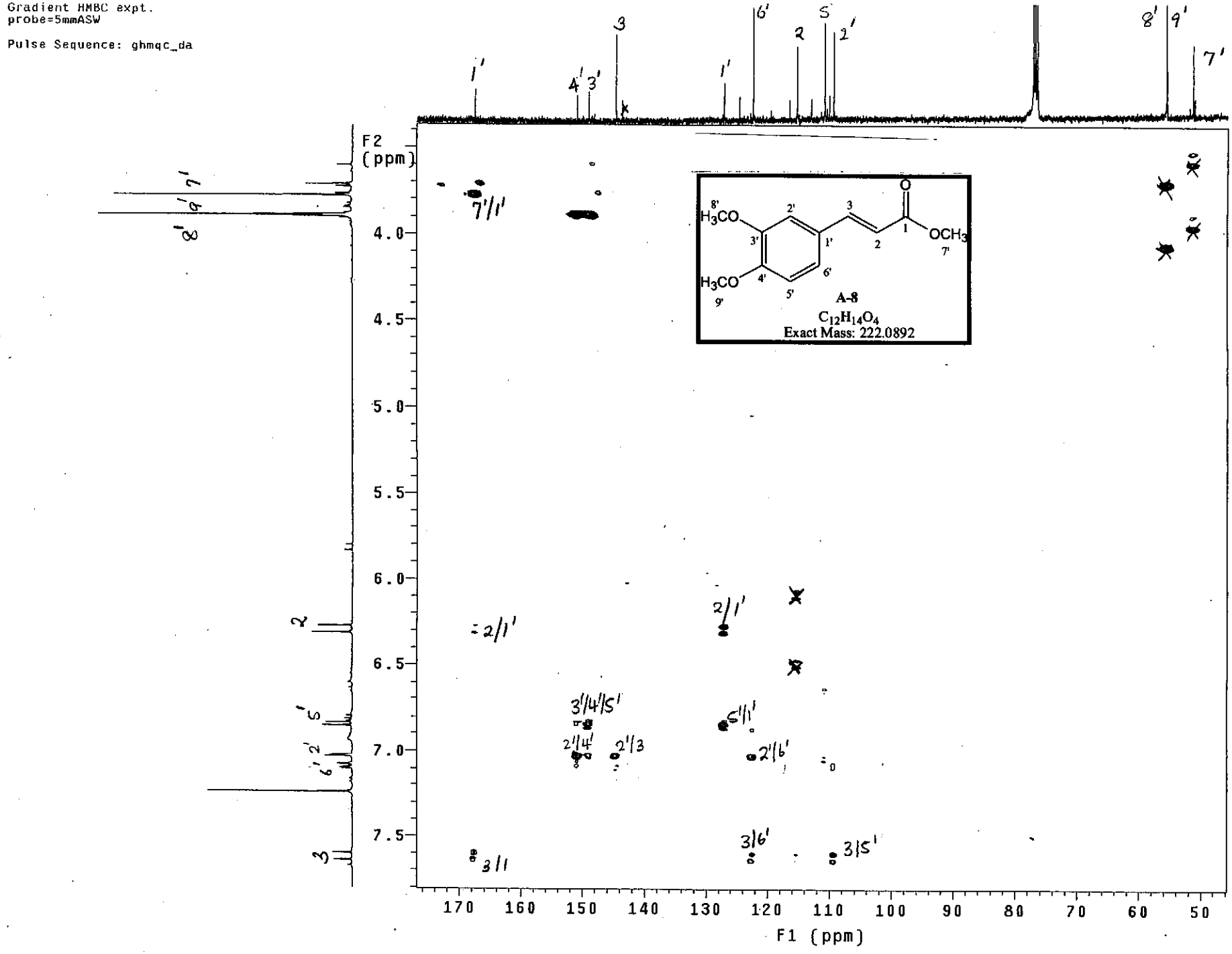
^{13}C NMR spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8



COSY spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-



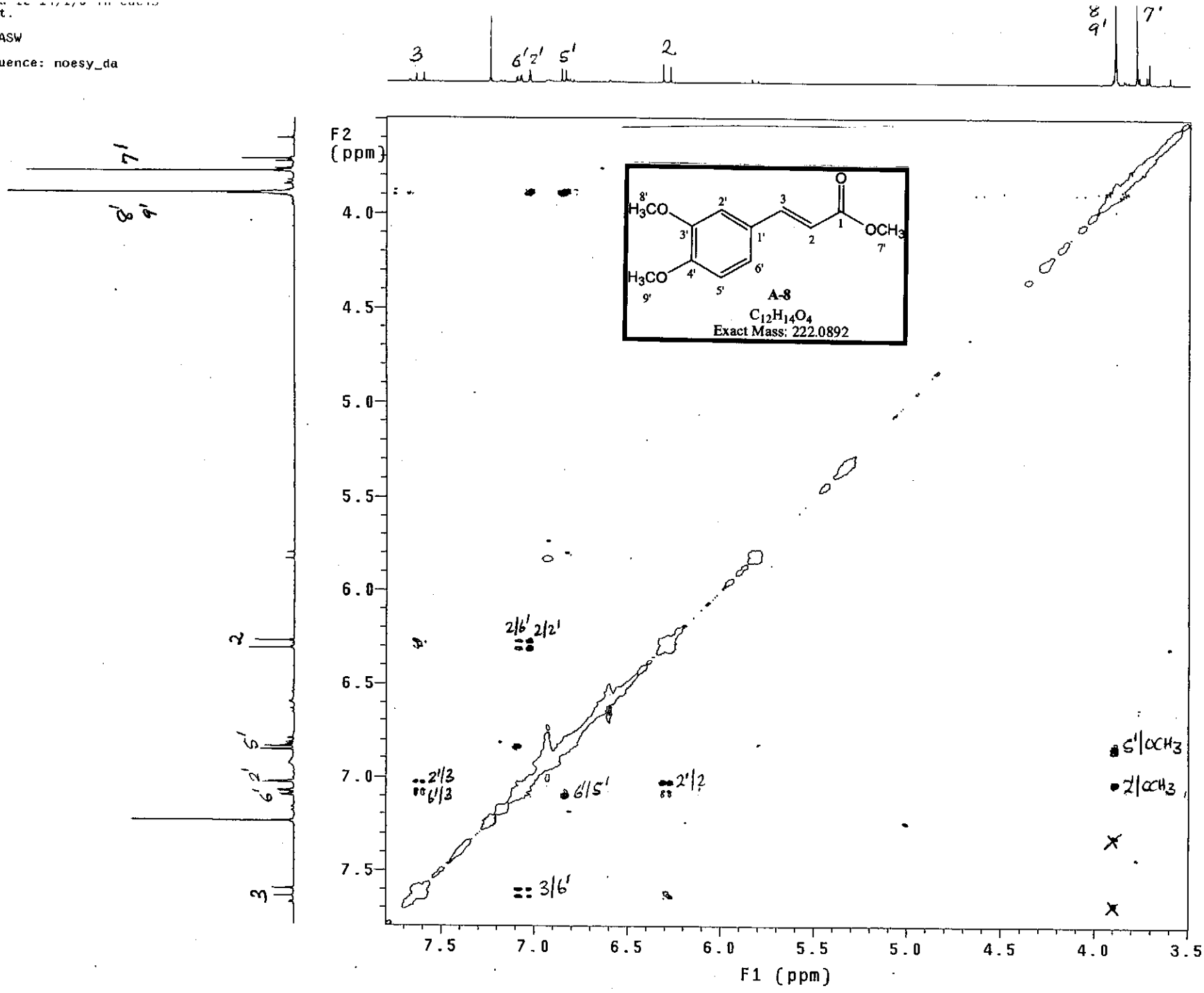
HSQC spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8



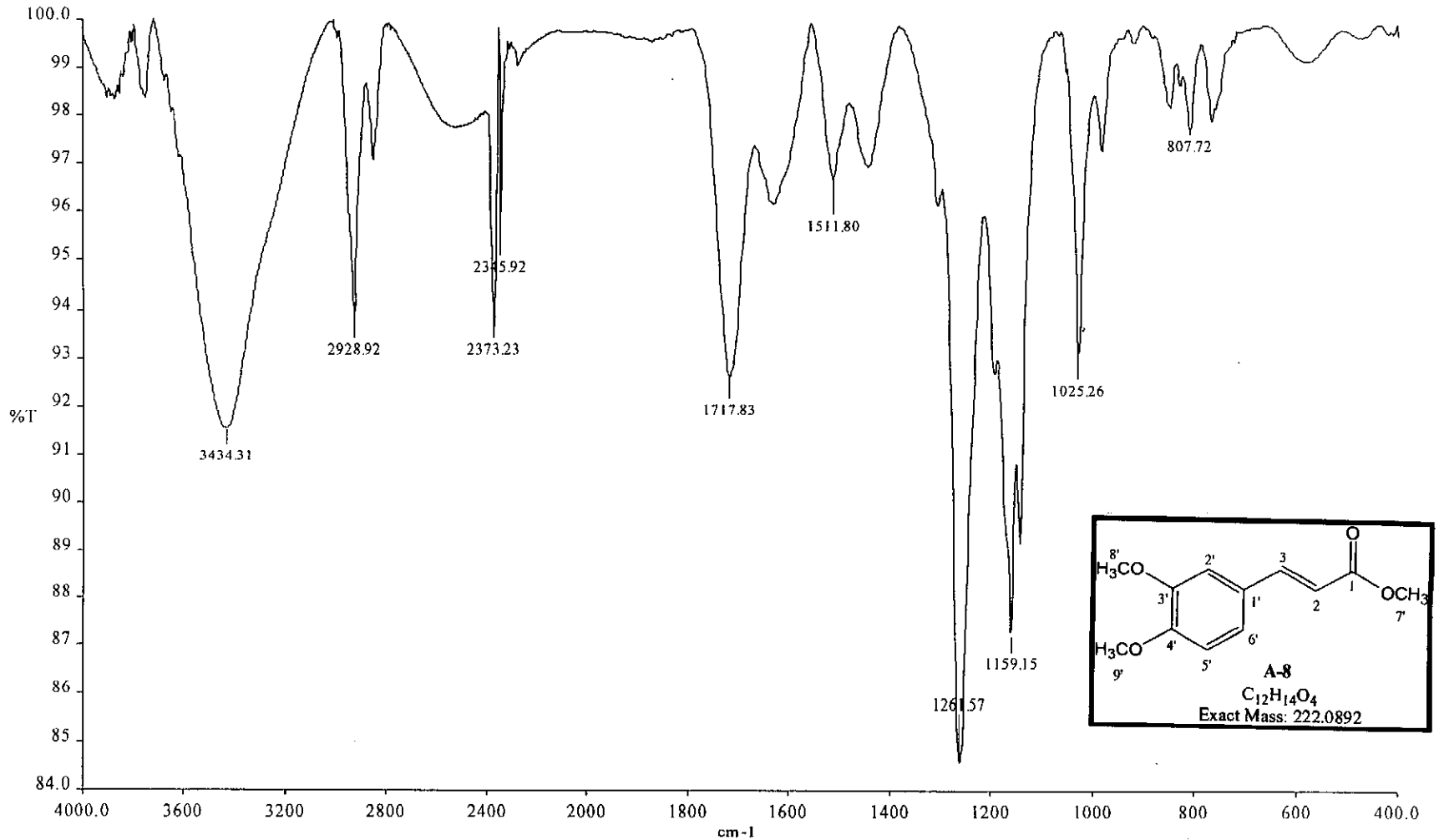
HMBC spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8

NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

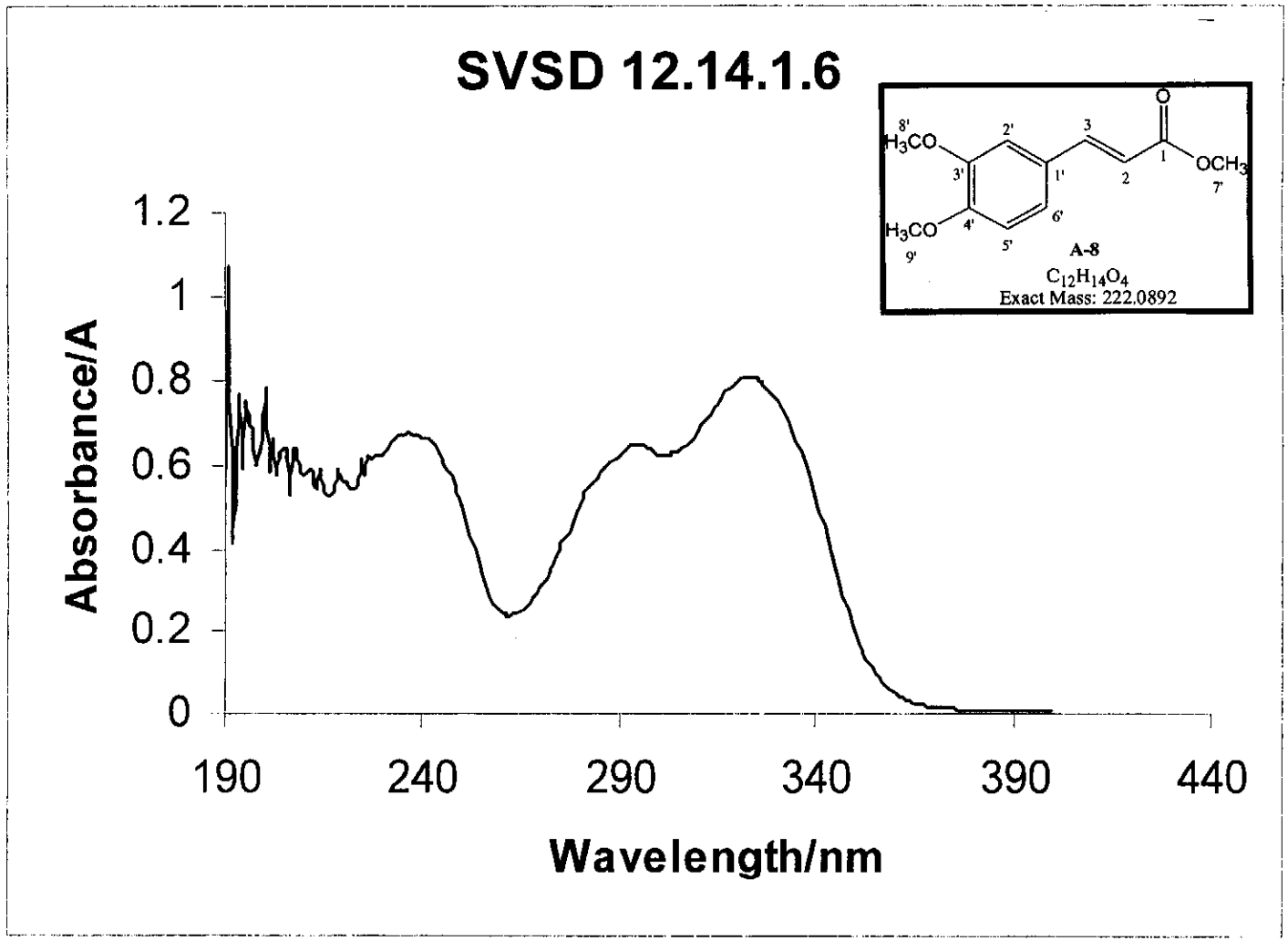


NOESY spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8

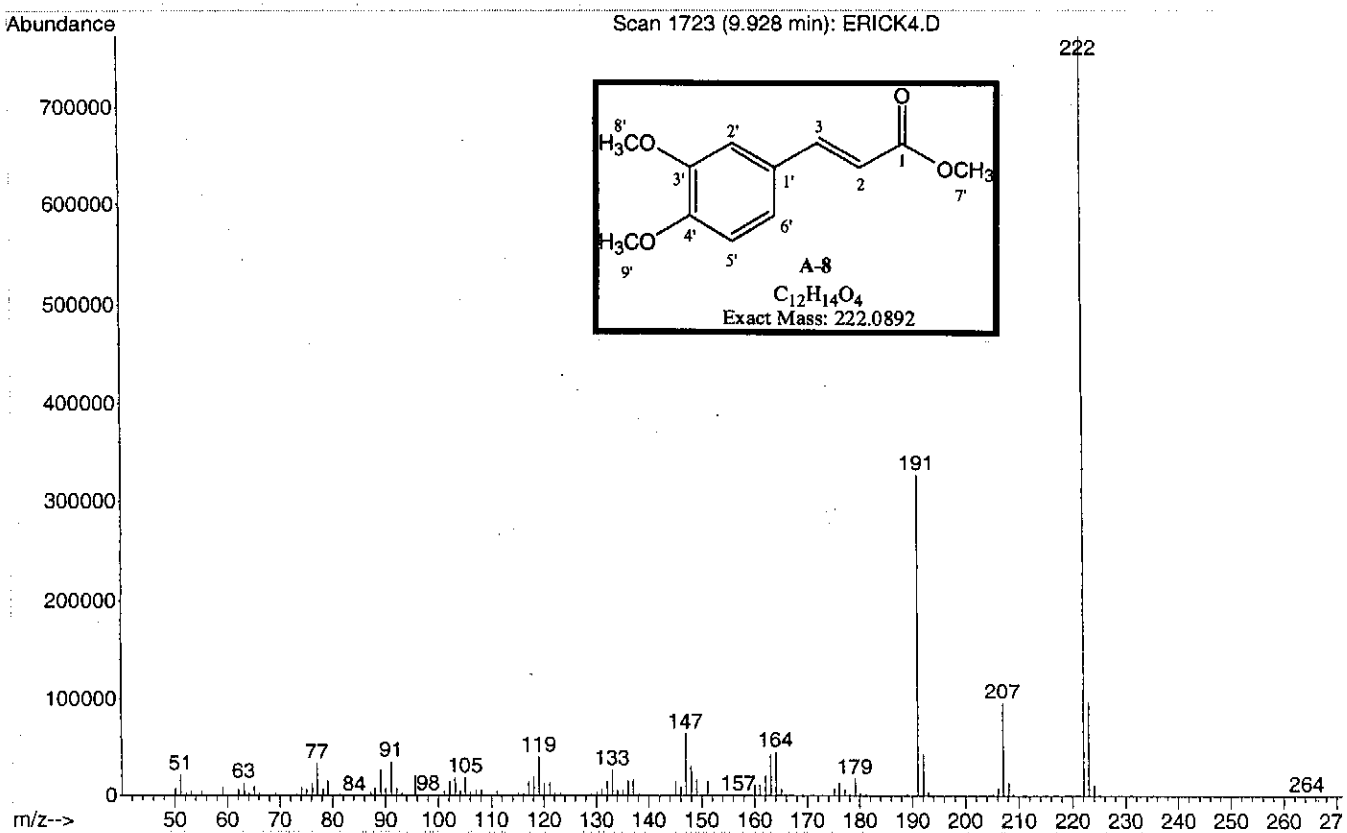


SVSD 12/14/1/6

IR spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8



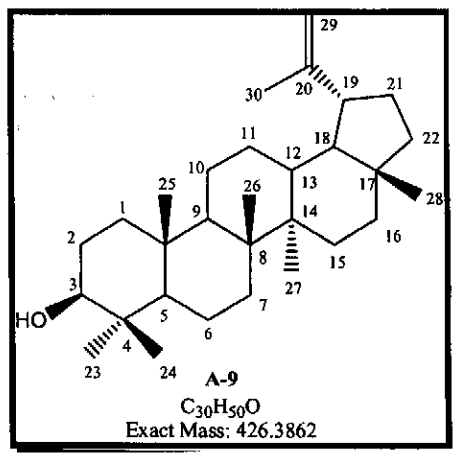
UV spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8



Mass spectrum of methyl-3-(3',4'-dimethoxyphenyl)-2-propenoate A8

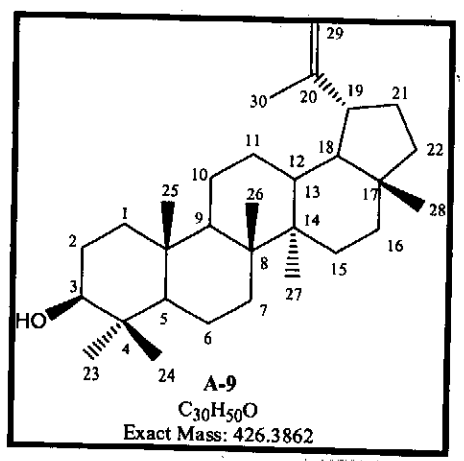
lupeol

Pulse Sequence: s2pul



^{1}H NMR spectrum of lupeol A9

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2895.544	7.240	10.7	40	601.848	1.505	9.8	79	423.663	1.059	6.3
2	1865.807	4.665	17.5	41	598.369	1.496	8.5	80	419.268	1.048	4.6
3	1863.426	4.659	19.8	42	595.072	1.488	7.6	81	411.211	1.028	5.4
4	1819.292	4.549	5.0	43	591.959	1.480	10.3	82	406.083	1.015	5.9
5	1818.010	4.546	14.9	44	589.395	1.474	12.0	83	401.688	1.004	150.0
6	1816.728	4.543	18.9	45	587.564	1.469	7.8	84	397.110	0.993	12.8
7	1815.630	4.540	18.2	46	584.817	1.462	6.7	85	394.363	0.986	7.4
8	1814.165	4.536	14.8	47	579.140	1.448	10.1	86	387.587	0.969	6.7
9	1812.883	4.533	5.7	48	576.576	1.442	11.4	87	385.023	0.963	7.8
10	1272.468	3.182	10.6	49	574.562	1.437	9.7	88	383.375	0.959	8.0
11	1267.340	3.169	9.2	50	571.998	1.430	8.2	89	380.628	0.952	7.9
12	1261.114	3.153	12.8	51	567.603	1.419	4.3	90	376.599	0.942	130.9
13	1256.169	3.141	11.4	52	561.010	1.403	9.9	91	367.809	0.920	105.5
14	949.245	2.373	4.4	53	558.630	1.397	8.9	92	367.259	0.918	107.4
15	943.934	2.360	7.4	54	553.685	1.384	17.2	93	361.033	0.903	5.6
16	938.074	2.346	7.7	55	552.770	1.382	18.9	94	352.609	0.882	8.5
17	932.946	2.333	4.3	56	545.444	1.364	53.3	95	348.214	0.871	11.0
18	927.086	2.318	4.1	57	543.979	1.360	54.9	96	342.171	0.856	5.2
19	759.156	1.898	4.8	58	538.669	1.347	16.9	97	339.607	0.849	6.0
20	757.508	1.894	4.1	59	533.358	1.334	34.8	98	335.395	0.839	5.2
21	754.578	1.887	5.6	60	529.329	1.324	8.4	99	321.477	0.804	105.9
22	746.154	1.866	5.8	61	525.300	1.313	10.2	100	305.179	0.763	107.9
23	662.281	1.656	97.2	62	522.004	1.305	15.8	101	294.008	0.735	142.4
24	661.731	1.655	97.1	63	519.440	1.299	9.1	102	268.553	0.671	6.4
25	652.209	1.631	22.0	64	518.158	1.296	7.8	103	266.721	0.667	8.1
26	650.927	1.628	21.1	65	515.961	1.290	6.2	104	257.565	0.644	9.9
27	647.264	1.618	15.7	66	514.862	1.287	5.8				
28	642.503	1.607	10.4	67	511.382	1.279	7.7				
29	638.657	1.597	8.8	68	510.284	1.276	7.7				
30	634.811	1.587	8.0	69	506.987	1.268	9.9				
31	631.332	1.579	7.7	70	505.705	1.264	10.5				
32	629.684	1.574	13.9	71	504.606	1.262	9.7				
33	626.204	1.566	12.1	72	494.351	1.236	29.3				
34	621.809	1.555	5.1	73	491.604	1.229	41.6				
35	617.597	1.544	7.6	74	484.096	1.210	8.8				
36	615.034	1.538	6.5	75	480.250	1.201	10.6				
37	613.752	1.535	6.4	76	471.460	1.179	11.8				
38	605.877	1.515	6.7	77	467.981	1.170	8.0				
39	604.412	1.511	5.6	78	459.740	1.150	9.5				



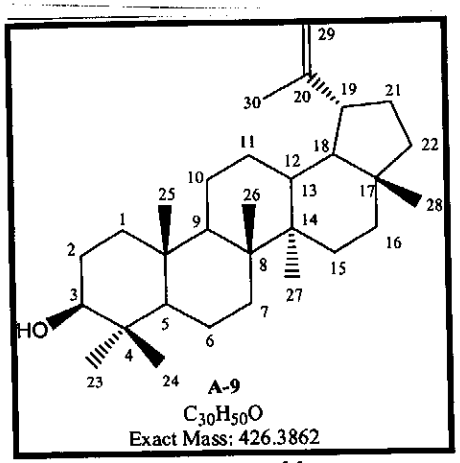
lupeol

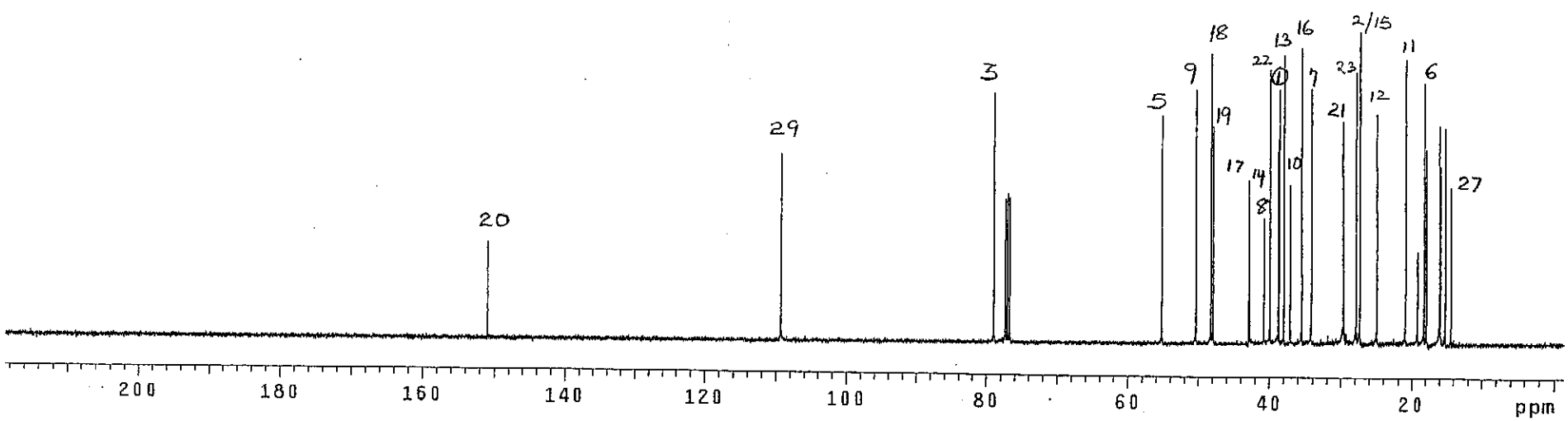
Pulse Sequence: s2pul

lupeol

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	15179.280	150.941	15.7
2	10992.904	109.312	30.2
3	7941.053	78.965	40.2
4	7775.490	77.319	23.2
5	7743.445	77.000	24.2
6	7711.401	76.681	23.4
7	5556.794	55.256	36.7
8	5068.498	50.401	41.0
9	4853.343	48.261	46.7
10	4822.824	47.958	35.0
11	4321.558	42.973	26.4
12	4303.246	42.791	23.8
13	4102.587	40.796	20.5
14	4020.187	39.976	44.1
15	3904.980	38.831	31.1
16	3888.958	38.671	41.1
17	3822.580	38.011	46.6
18	3734.076	37.131	26.0
19	3575.380	35.553	47.6
20	3443.387	34.241	41.2
21	2997.817	29.810	36.0
22	2984.847	29.681	12.2
23	2812.417	27.966	43.6
24	2756.721	27.413	50.0
25	2753.669	27.382	41.4
26	2524.017	25.099	37.0
27	2101.336	20.895	45.8
28	1938.825	19.279	15.2
29	1839.640	18.293	42.2
30	1808.358	17.982	31.5
31	1619.144	16.101	35.2
32	1603.884	15.949	28.8
33	1543.610	15.349	34.8
34	1460.447	14.523	25.5

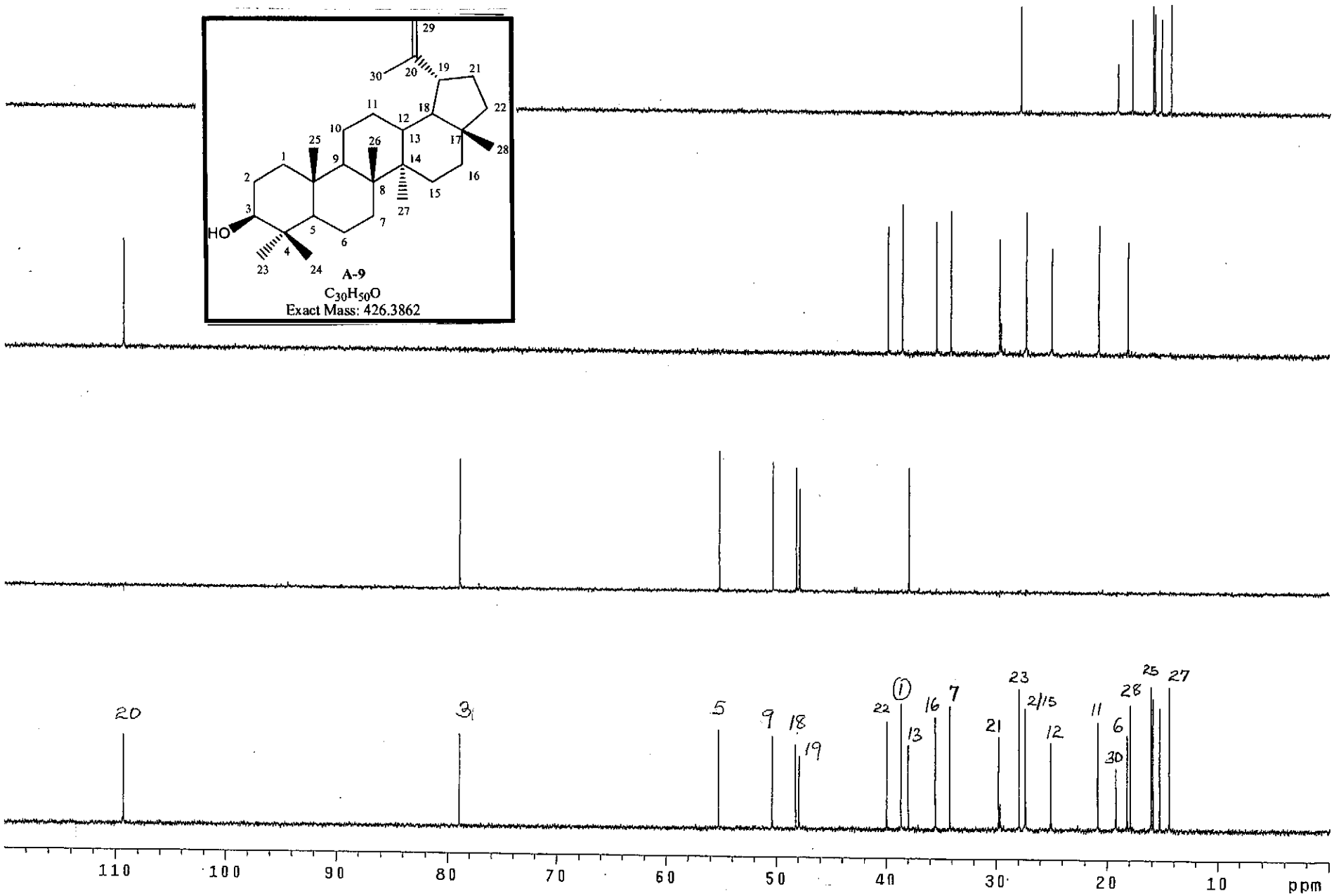




^{13}C NMR spectrum of lupeol A9

lupeol

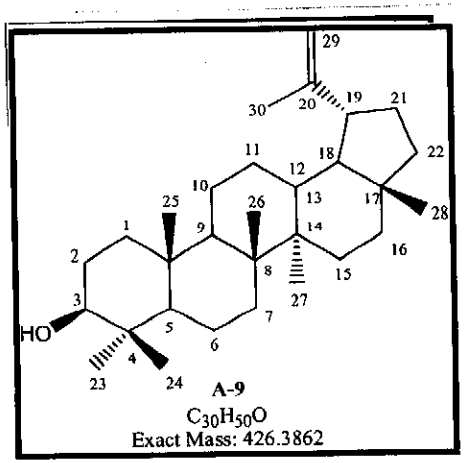
Pulse Sequence: dept



DEPT spectrum of lupeol A9

lupeol
1H Cosy-90
probe=5mmASW

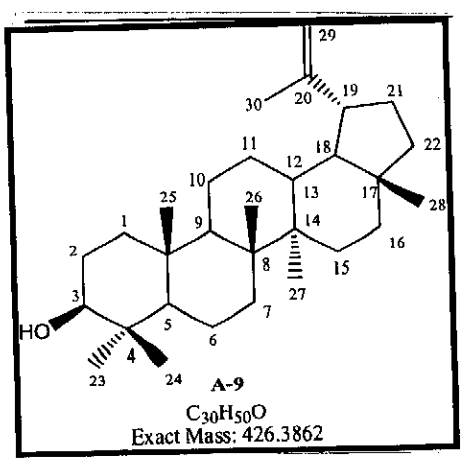
Pulse Sequence: relayh



COSY spectrum of lupeol A9

lupeol
Gradient HSQC expt.
with mult.editing
probe=5mmASW

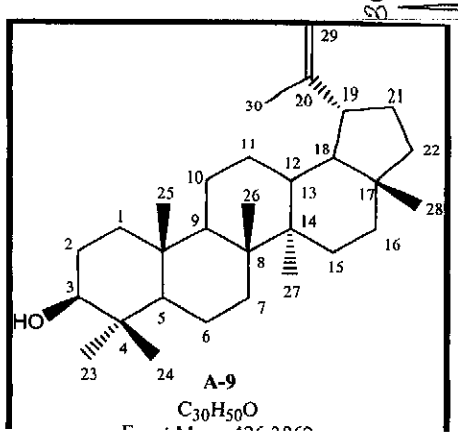
Pulse Sequence: ghsqc_da



HSQC spectrum of lupeol A9

lupeol
Gradient HSQC expt.
with mult.editing
probe=5mmASW

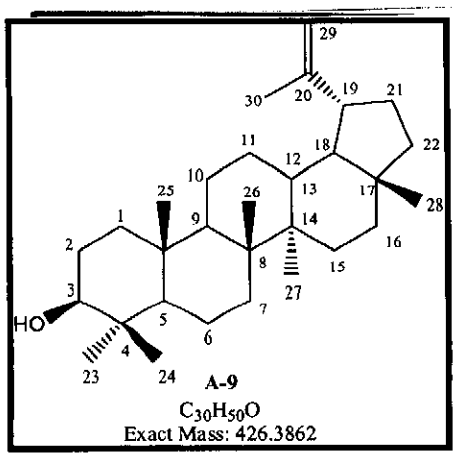
Pulse Sequence: ghsqc_da



HSQC spectrum of lupeol A9

lupeol
Gradient HMBC expt.
probe=5mmASW

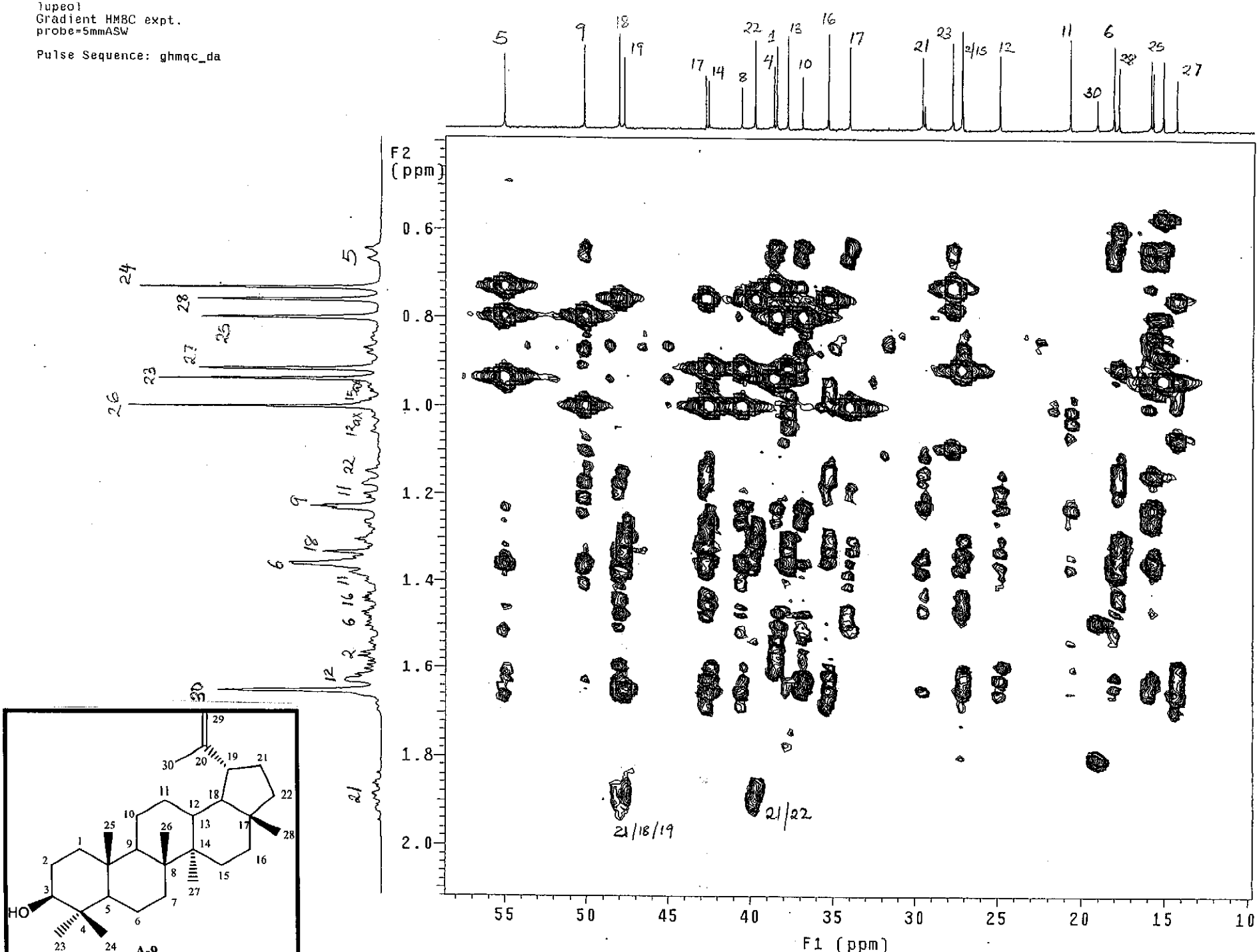
Pulse Sequence: ghmqc_da



HMBC spectrum of lupeol A9

lupeol
Gradient HMBC expt.
probe=5mmASW

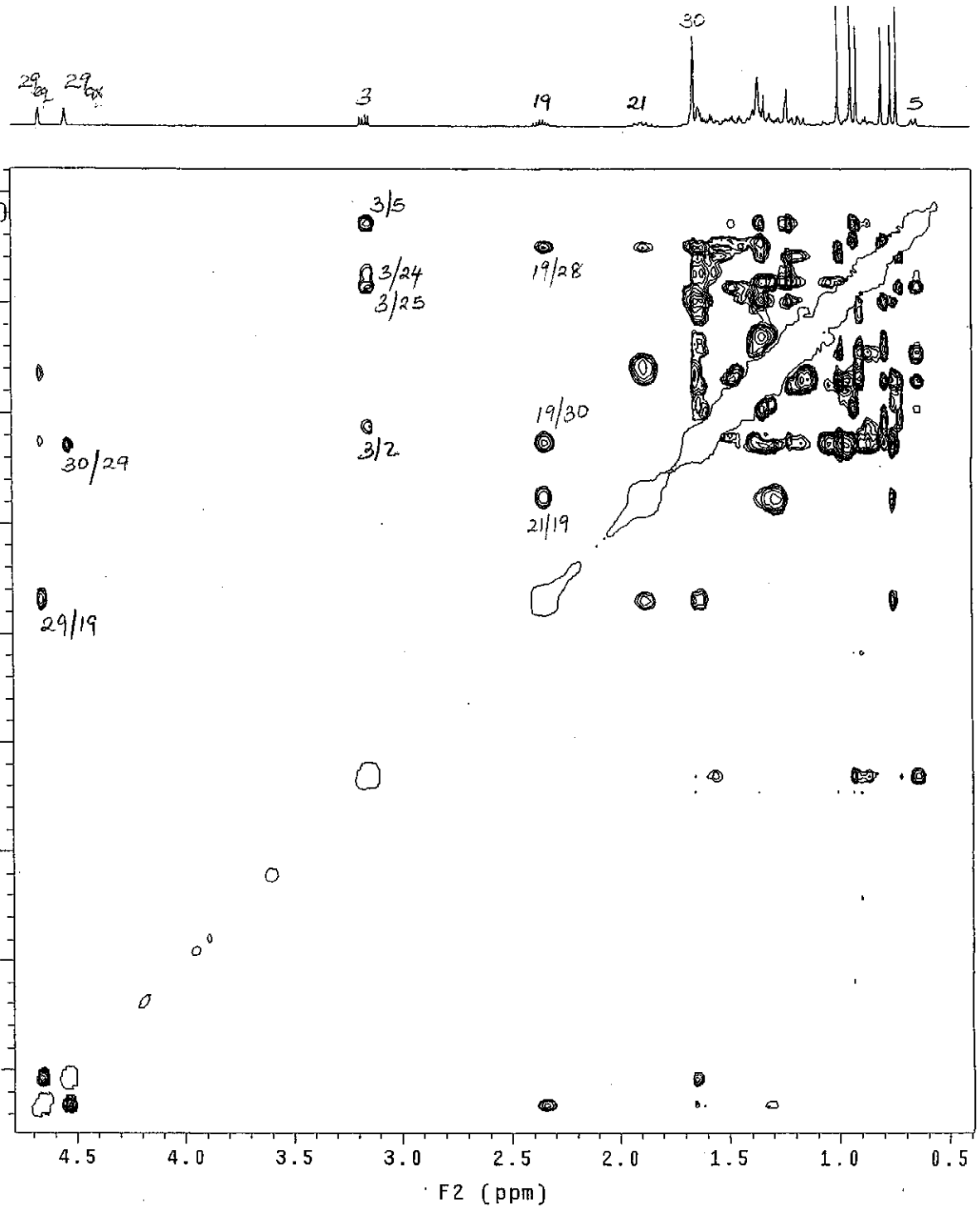
Pulse Sequence: ghmqc_da

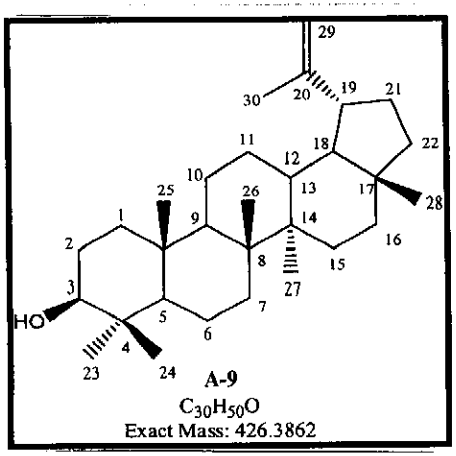


EXPANDED HMBC spectrum of lupeol A9

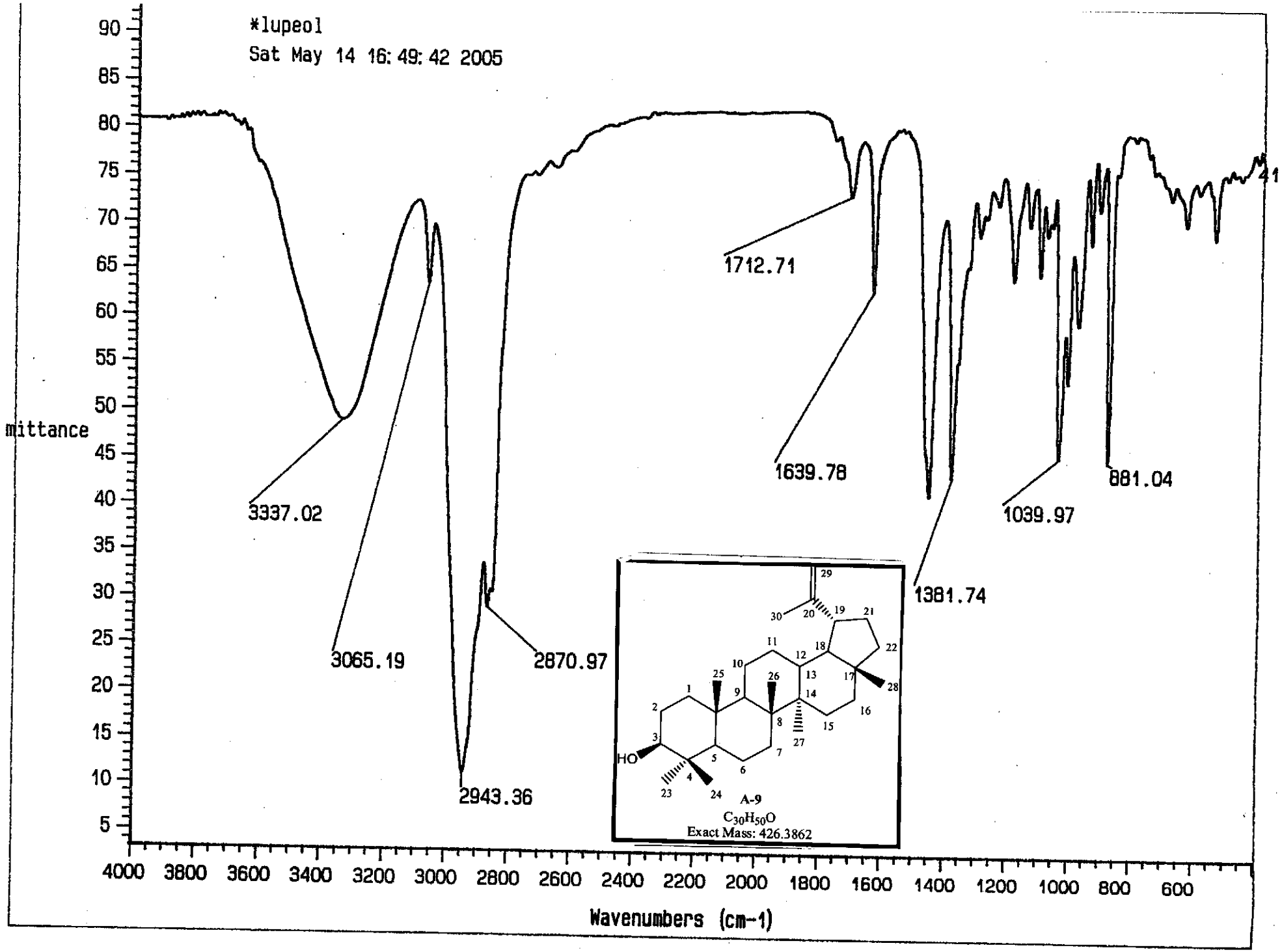
lupeol
NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da





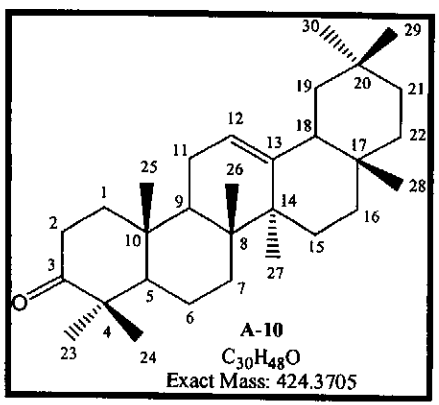
NOESY spectrum of lupeol A9



IR spectrum of lupeol A9

hsv682.svml 68.2 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul



^{1}H NMR spectrum of 12-oleanene-3-one A10

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2896.652	7.243	5.6	40	705.509	1.764	5.1	79	483.922	1.210	8.7
2	2895.553	7.240	124.5	41	706.058	1.765	5.1	80	481.908	1.205	9.8
3	2077.697	5.195	6.2	42	701.663	1.754	5.1	81	477.330	1.194	6.5
4	2074.035	5.186	10.5	43	671.630	1.679	7.3	82	474.216	1.186	8.1
5	2070.372	5.177	6.2	44	658.261	1.646	18.7	83	471.286	1.178	5.3
6	1028.549	2.572	3.7	45	652.218	1.631	7.2	84	448.578	1.122	78.2
7	1021.224	2.553	4.3	46	646.907	1.618	7.2	85	443.084	1.108	5.2
8	1017.378	2.544	4.3	47	644.527	1.612	8.3	86	440.704	1.102	7.6
9	1012.617	2.532	6.0	48	640.681	1.602	6.6	87	436.858	1.092	6.9
10	1010.053	2.526	4.6	49	617.973	1.545	15.4	88	433.562	1.084	11.8
11	1005.292	2.514	6.3	50	613.944	1.535	14.4	89	429.716	1.074	110.2
12	1001.446	2.504	6.9	51	603.140	1.508	14.2	90	423.123	1.058	17.2
13	994.304	2.486	5.8	52	600.759	1.502	9.4	91	420.193	1.051	84.6
14	951.818	2.380	5.1	53	596.547	1.492	9.1	92	413.967	1.035	114.8
15	948.155	2.371	6.4	54	594.716	1.487	10.0	93	409.572	1.024	9.2
16	945.042	2.363	6.7	55	592.335	1.481	13.3	94	404.994	1.013	17.7
17	941.379	2.354	6.3	56	590.687	1.477	10.4	95	399.866	1.000	100.8
18	936.069	2.341	4.1	57	588.123	1.471	7.8	96	393.456	0.984	10.1
19	932.223	2.331	4.7	58	583.545	1.459	8.7	97	391.625	0.979	8.7
20	929.110	2.323	4.3	59	576.403	1.441	5.5	98	383.567	0.959	4.5
21	925.447	2.314	4.0	60	573.106	1.433	5.7	99	381.004	0.953	5.3
22	854.759	2.137	4.1	61	566.697	1.417	10.6	100	376.609	0.942	8.7
23	835.164	2.088	9.5	62	562.485	1.406	10.9	101	367.818	0.920	16.5
24	792.129	1.981	6.4	63	553.511	1.384	13.1	102	357.563	0.894	4.9
25	787.551	1.969	7.4	64	549.849	1.375	15.9	103	349.322	0.873	3.8
26	782.973	1.958	5.4	65	543.439	1.359	8.4	104	340.715	0.852	200.0
27	779.310	1.949	10.4	66	540.876	1.352	9.5	105	333.573	0.834	20.0
28	776.380	1.941	6.6	67	537.396	1.344	8.5	106	327.530	0.819	110.2
29	774.182	1.936	5.7	68	534.283	1.336	8.0	107	323.501	0.809	12.3
30	767.773	1.920	10.4	69	527.141	1.318	10.4	108	315.443	0.789	4.8
31	765.026	1.913	7.9	70	523.845	1.310	11.7	109	313.612	0.784	5.1
32	760.448	1.901	4.9	71	521.098	1.303	11.8	110	311.598	0.779	5.6
33	756.602	1.892	8.4	72	518.351	1.296	8.7	111	309.400	0.774	4.8
34	753.122	1.883	7.8	73	513.589	1.284	7.9	112	290.171	0.726	7.6
35	749.643	1.874	8.4	74	511.392	1.279	7.1				
36	747.079	1.868	7.9	75	507.546	1.269	7.9				
37	743.417	1.859	6.1	76	501.686	1.254	6.4				
38	739.937	1.850	5.4	77	492.163	1.231	49.4				
39	736.091	1.841	4.9	78	487.036	1.218	12.7				

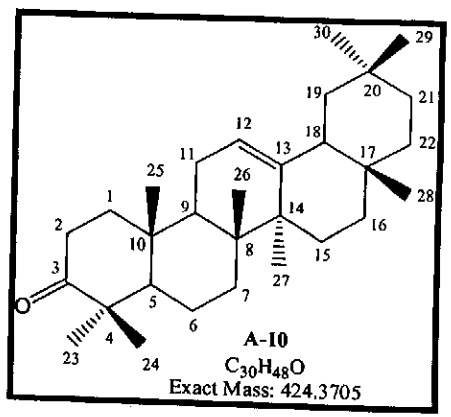
hsv682.svm1 68.2 in cdcl3
probe=5mmASW

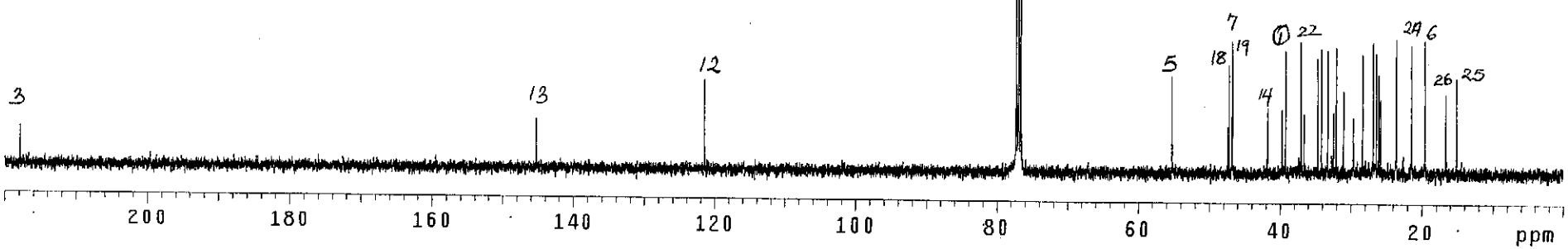
Pulse Sequence: s2pul

csv682.svm1 68.2 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	21913.617	217.906	6.2
2	14608.951	145.269	8.1
3	12216.909	121.483	14.5
4	7774.782	77.311	149.3
5	7764.070	77.205	9.4
6	7742.647	76.992	150.0
7	7711.335	76.680	143.6
8	5558.251	55.270	15.7
9	4772.989	47.462	7.7
10	4754.861	47.282	17.7
11	4710.365	46.839	21.2
12	4701.302	46.749	19.1
13	4206.908	41.833	10.7
14	3998.439	39.760	10.4
15	3949.823	39.276	19.9
16	3728.170	37.072	21.2
17	3686.147	36.655	9.8
18	3488.389	34.688	18.7
19	3438.950	34.196	20.3
20	3349.959	33.312	20.0
21	3268.384	32.500	10.0
22	3232.128	32.140	20.5
23	3125.010	31.075	13.5
24	2984.932	29.682	9.2
25	2856.389	28.404	19.4
26	2704.775	26.896	21.1
27	2659.456	26.445	19.7
28	2624.024	26.093	16.0
29	2600.128	25.855	12.1
30	2379.299	23.659	19.0
31	2376.003	23.627	21.8
32	2160.942	21.488	20.8
33	1973.896	19.628	21.4
34	1679.732	16.703	13.0
35	1529.766	15.212	15.6

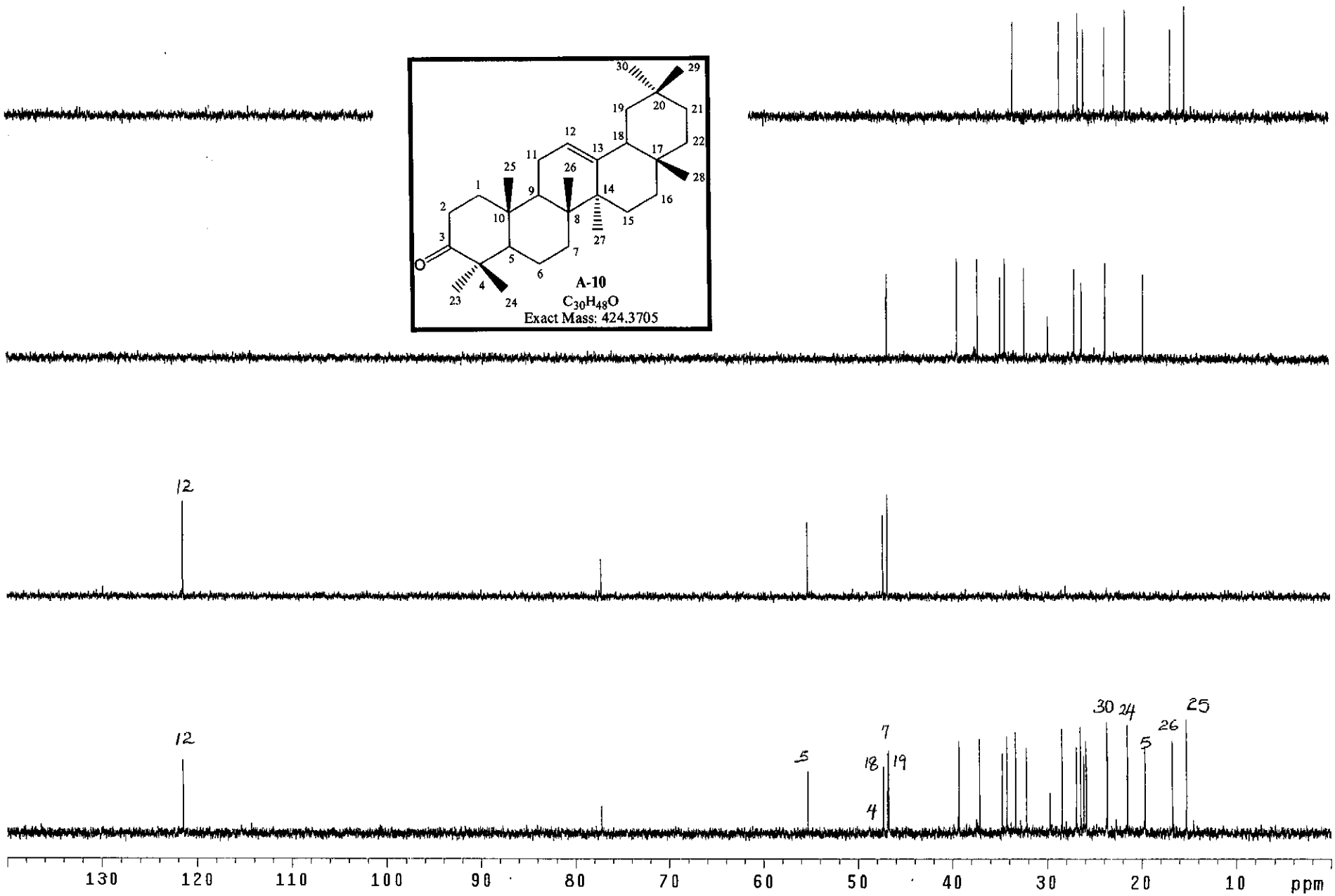




^{13}C NMR spectrum of 12-oleanene-3-one A 10

dsv682.svml 68.2 in cdcl3
probe=5mmASW

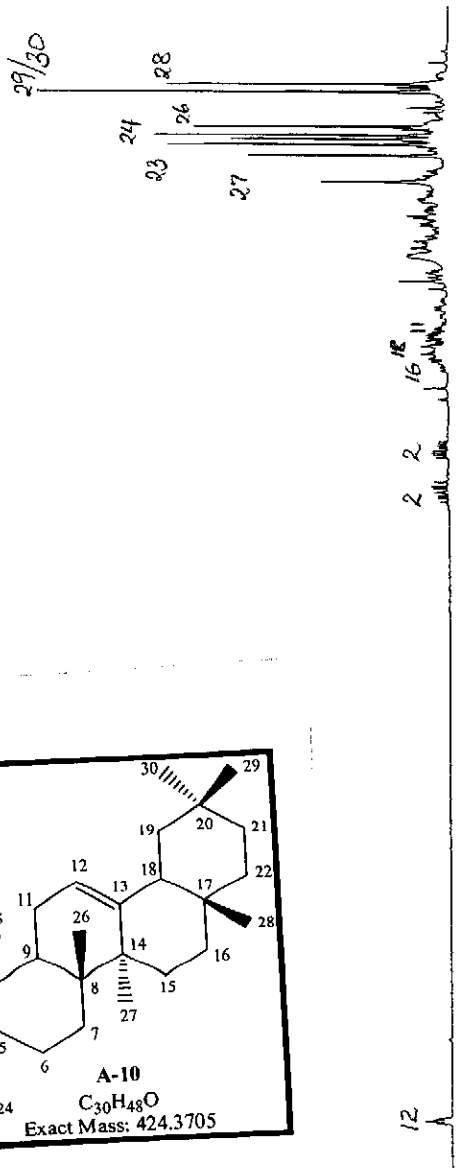
Pulse Sequence: dept

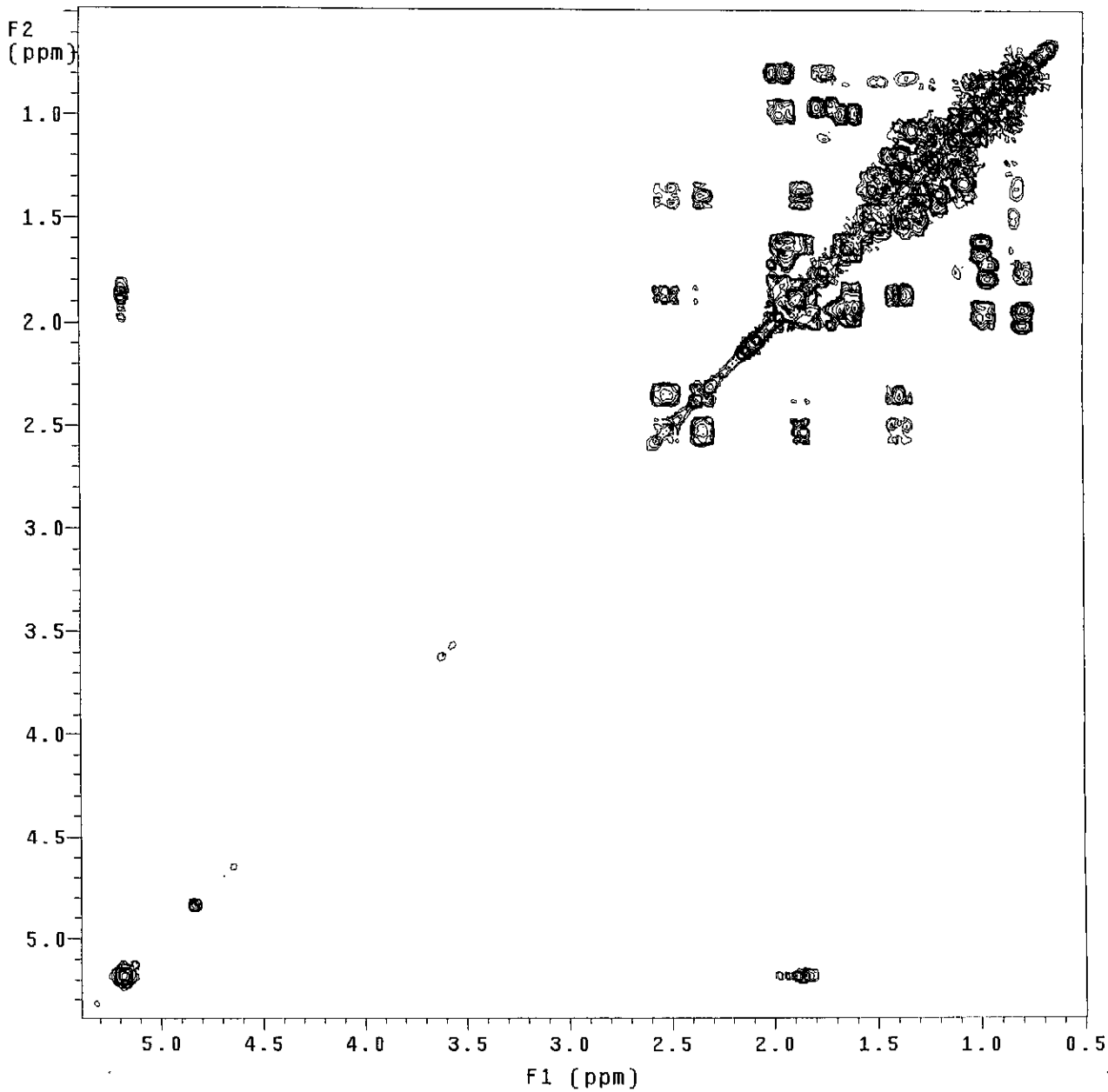


DEPT spectrum of 12-oleanene-3-one A 10

cysv682.svml 68.2 in cdcl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

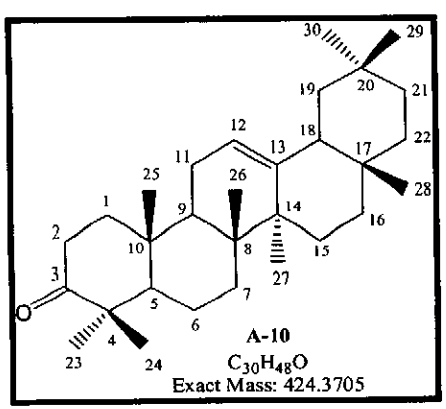




COSY spectrum of 12-oleanene-3-one A 10

cysv682.svml 68.2 in cdcl3
1H Cosy-90
probe=5mmASW

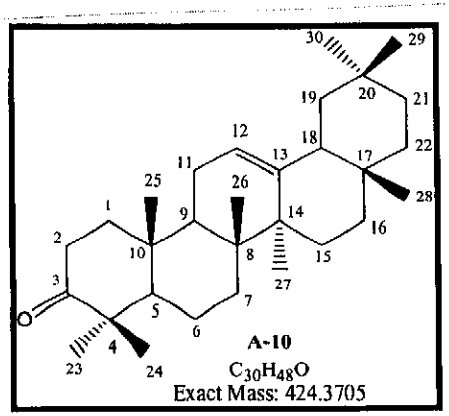
Pulse Sequence: relayh

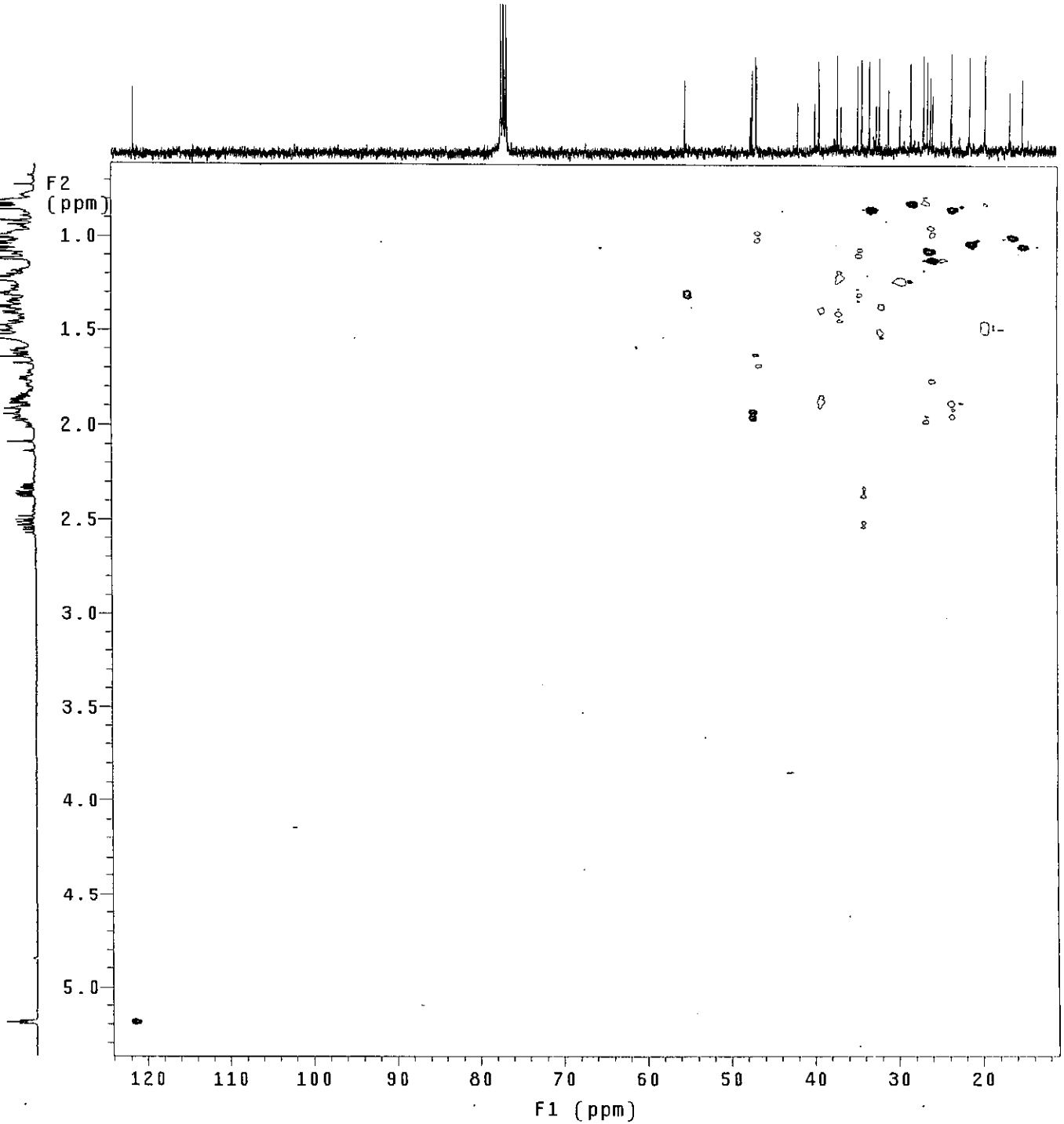


Expanded COSY spectrum of 12-oleanene-3-one A 10

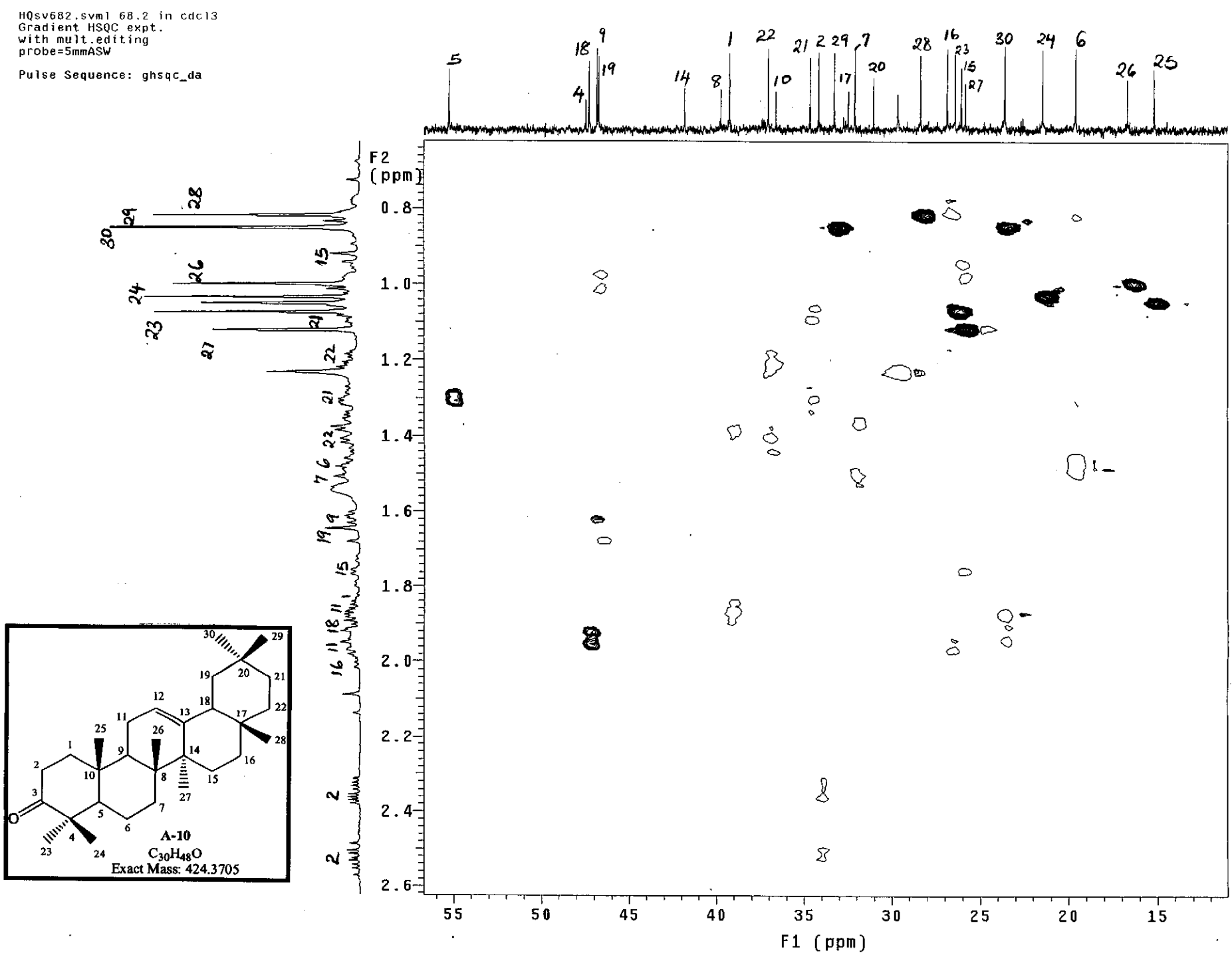
HQsv682.svml 68.2 in cdcl3
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da

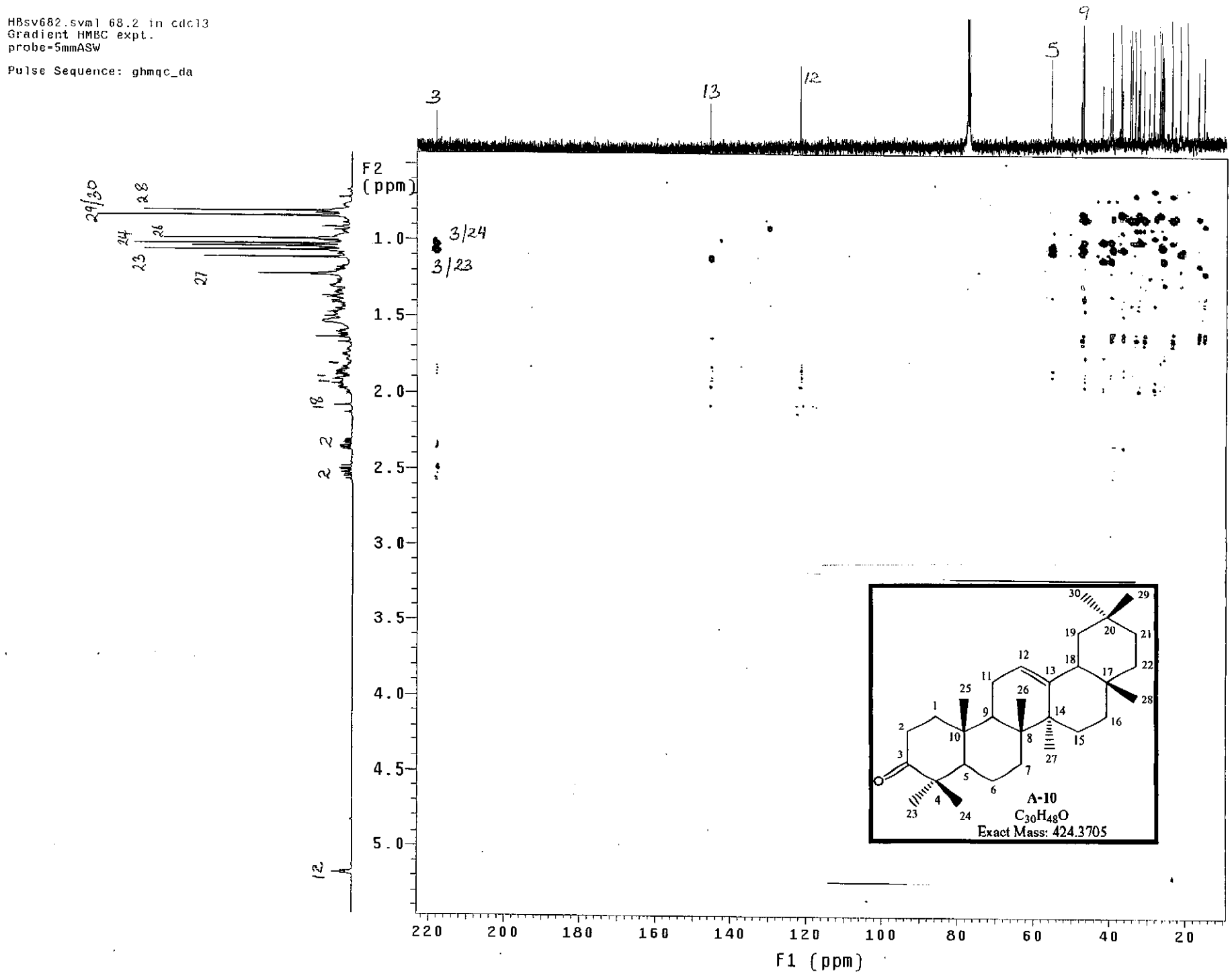




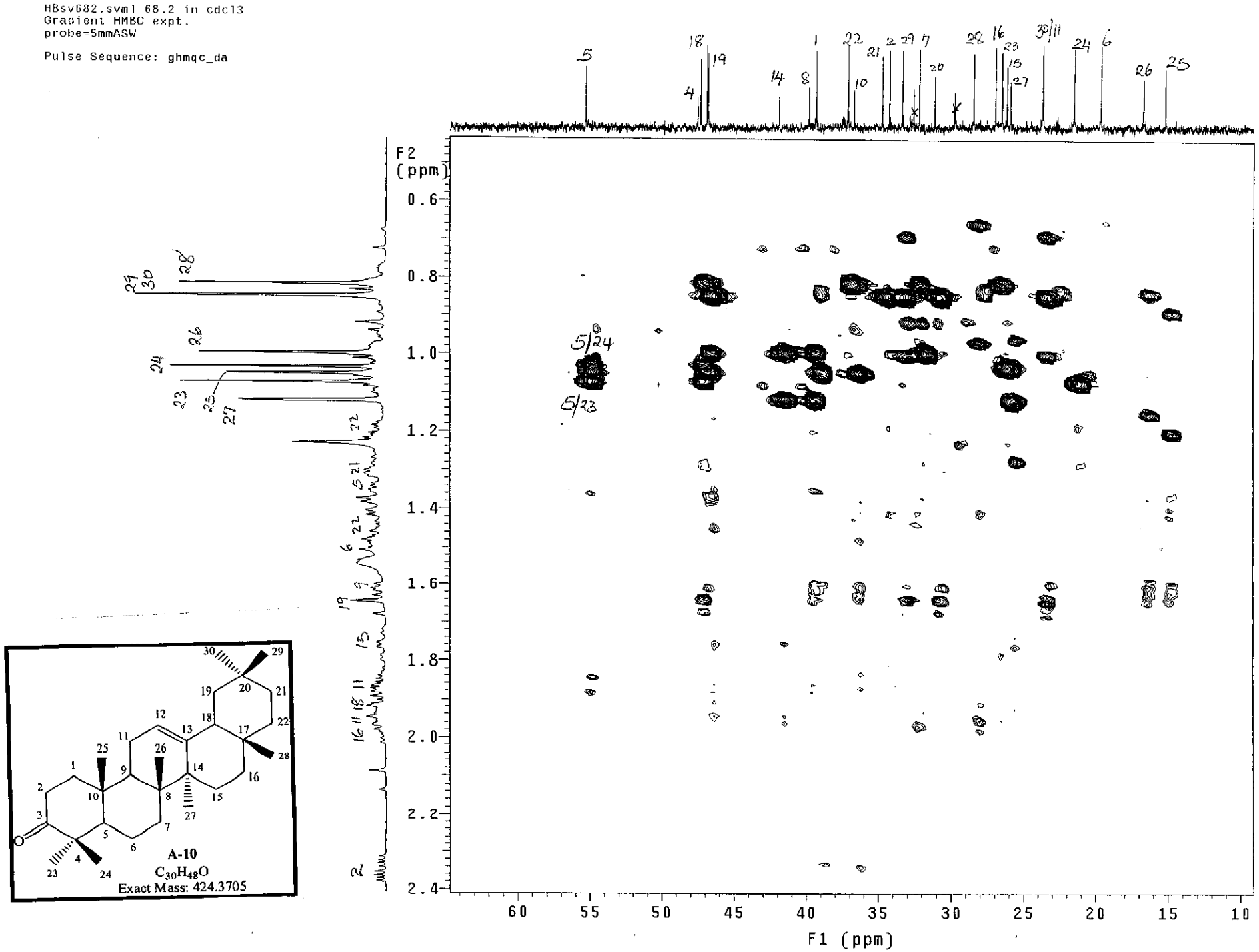
HSQC spectrum of 12-oleanene-3-one A 10



Expanded HSQC spectrum of 12-oleanene-3-one A 10



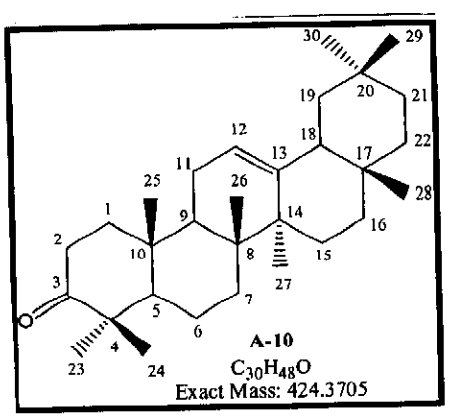
HMBC spectrum of 12-oleanene-3-one A 10

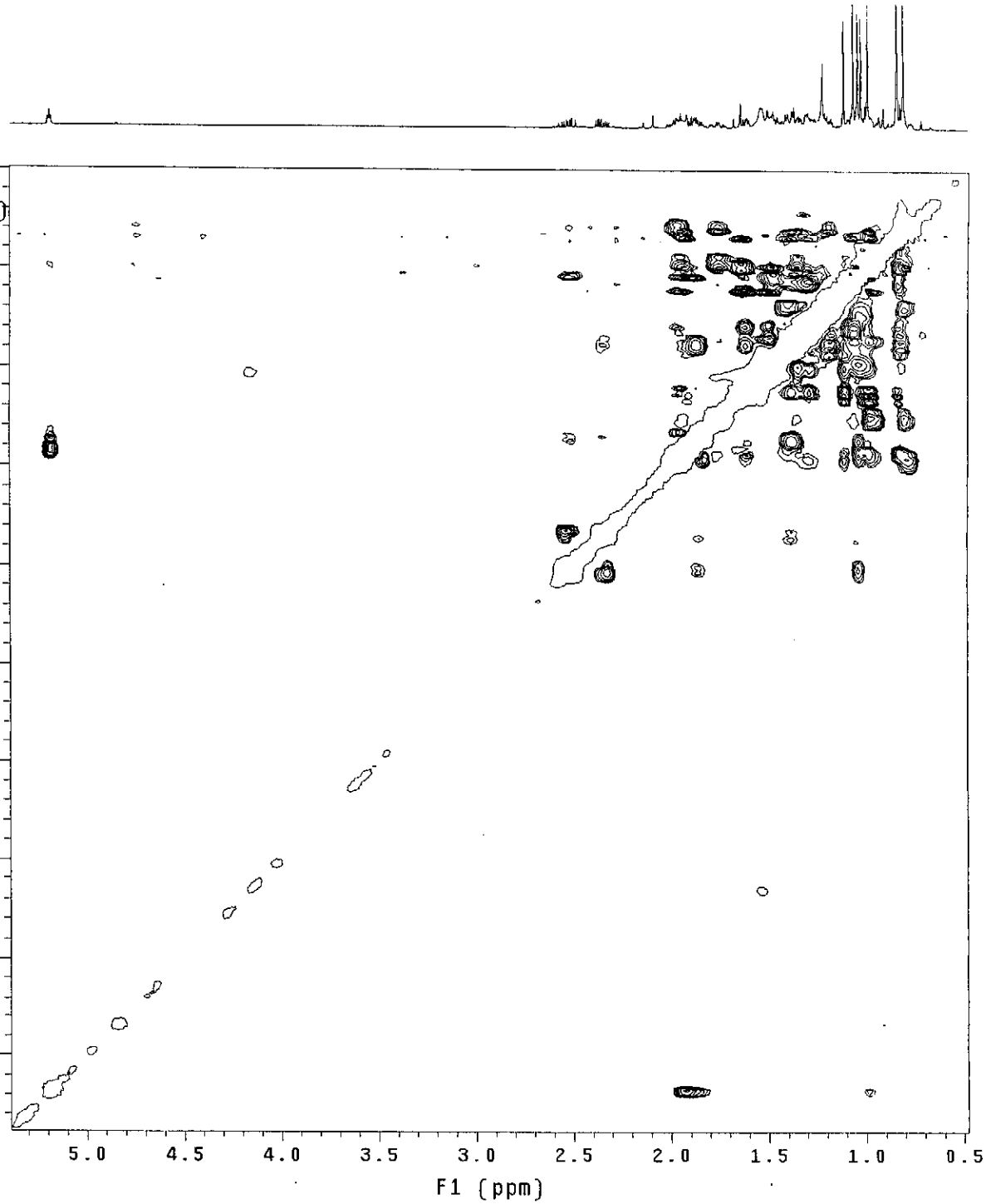


Expanded HMBC spectrum of 12-oleanene-3-one A1o

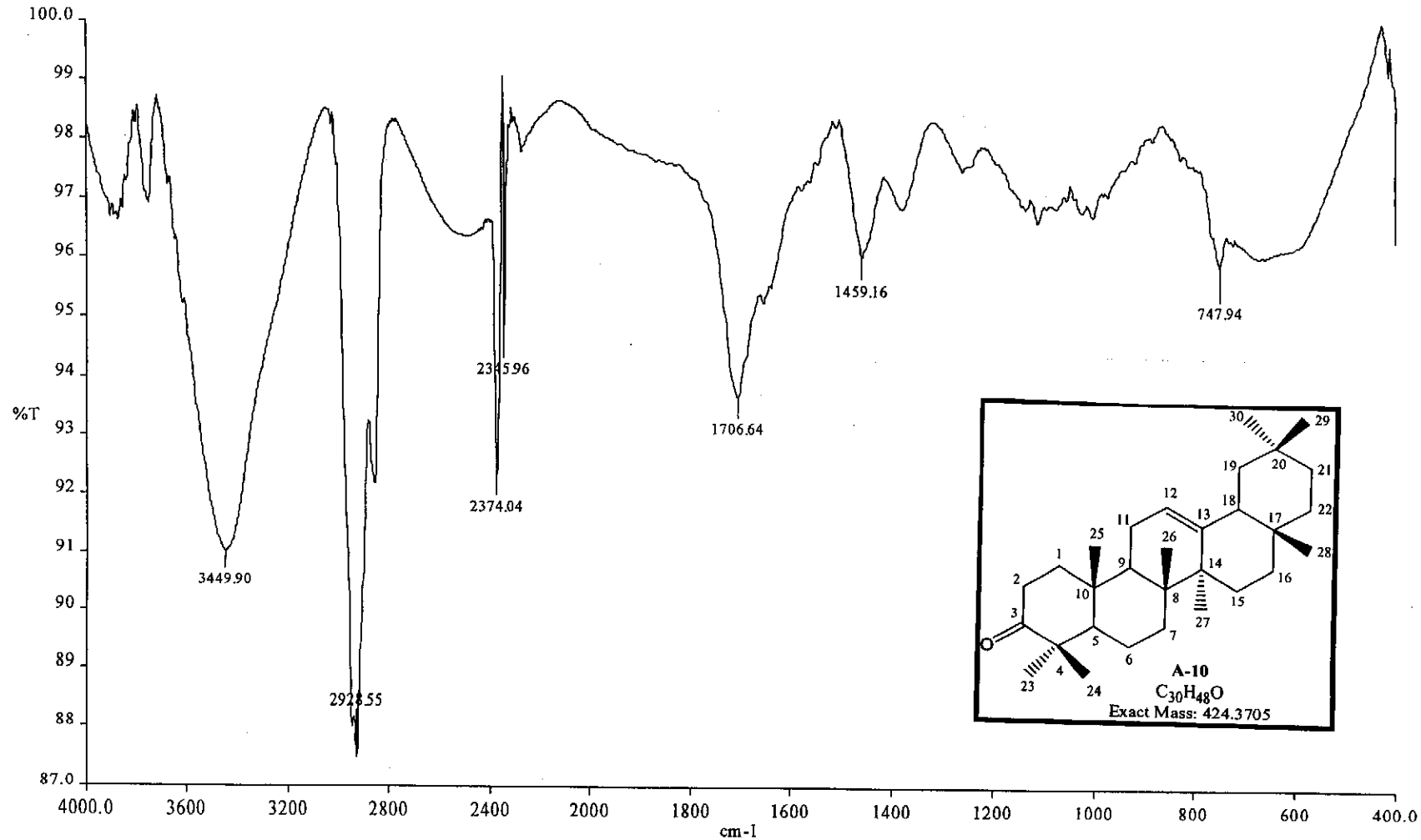
NOsv682.svml 68.2 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da



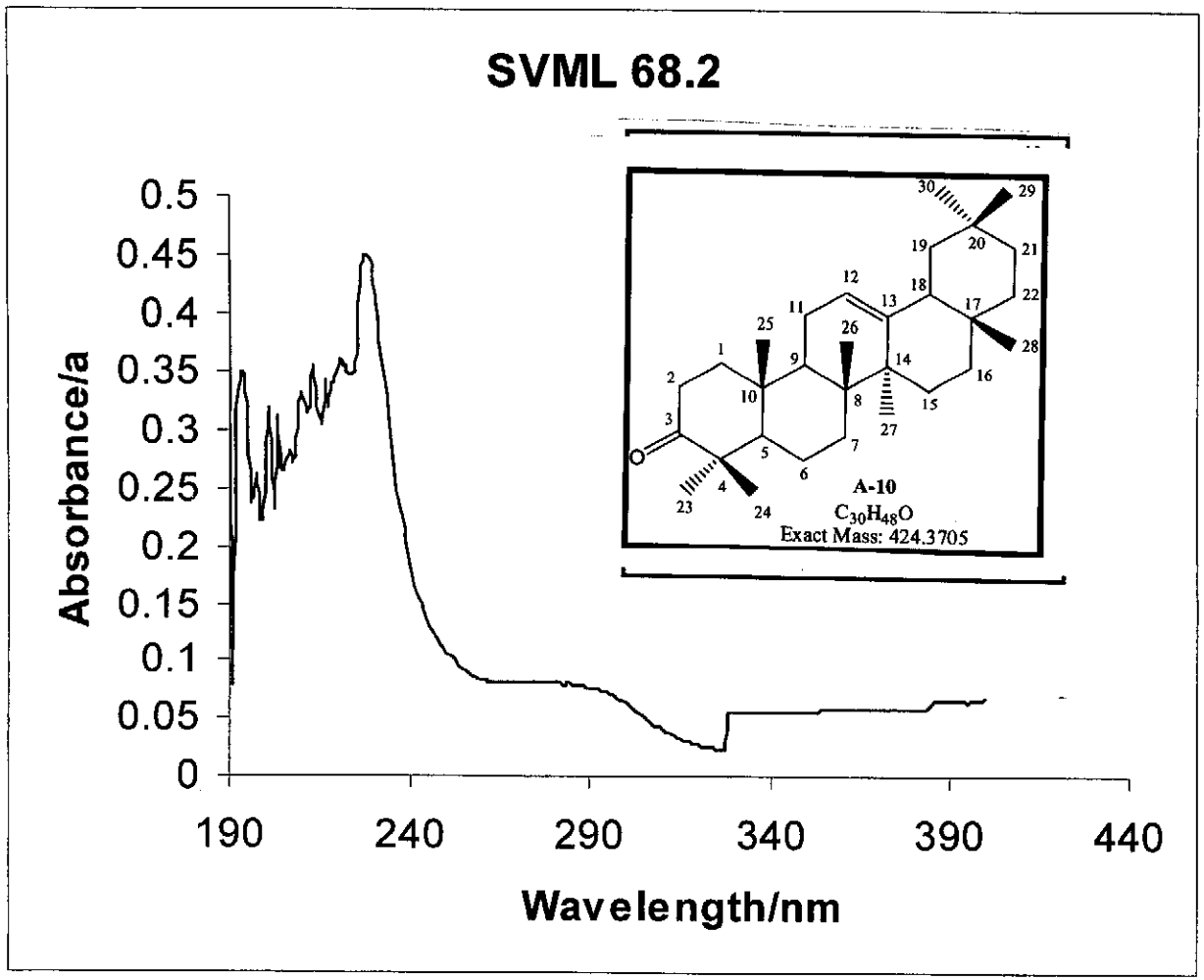


NOESY spectrum of 12-oleanene-3-one A-10

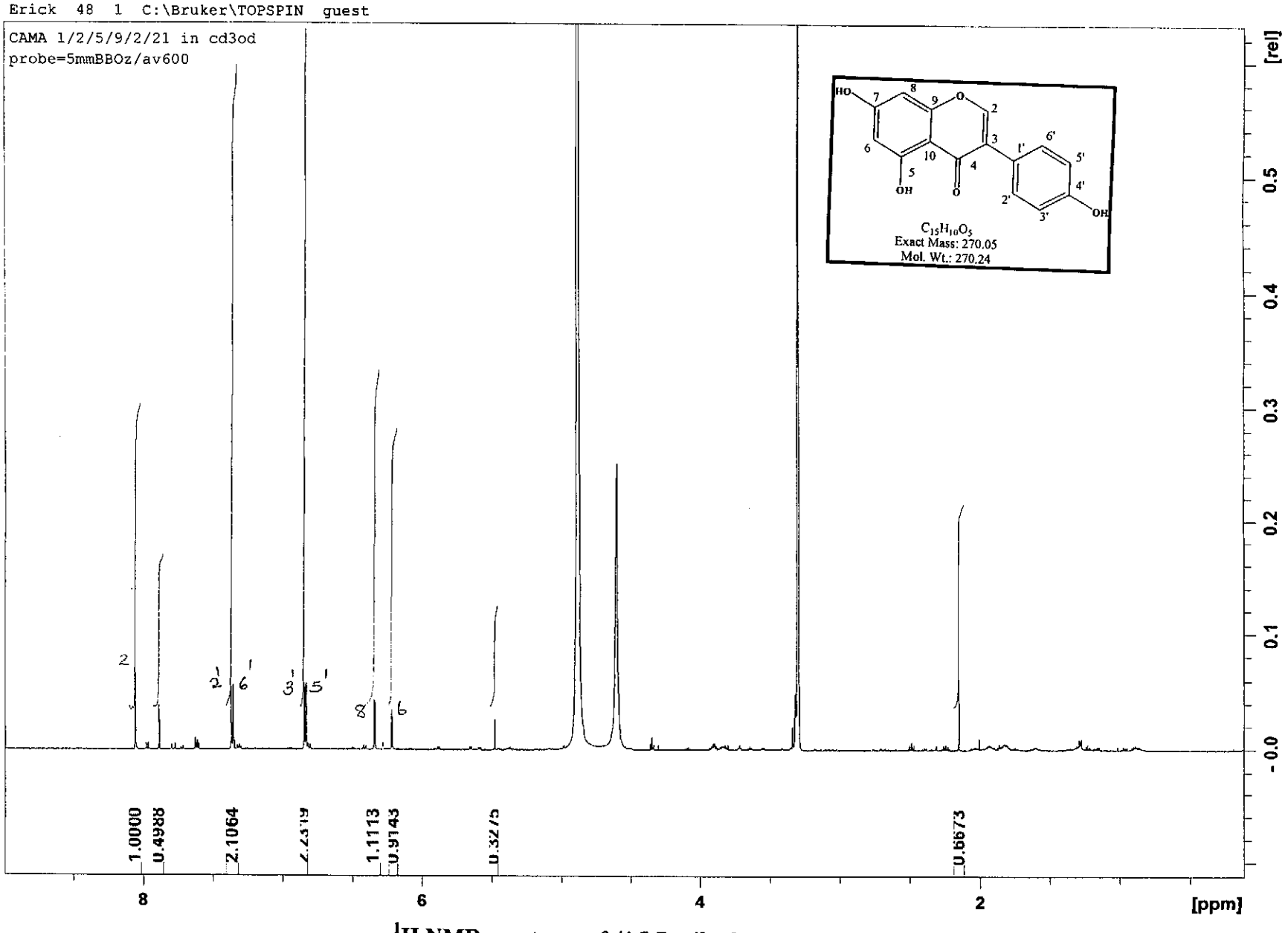


SVML 68.2

IR spectrum of 12-oleanene-3-one A 10

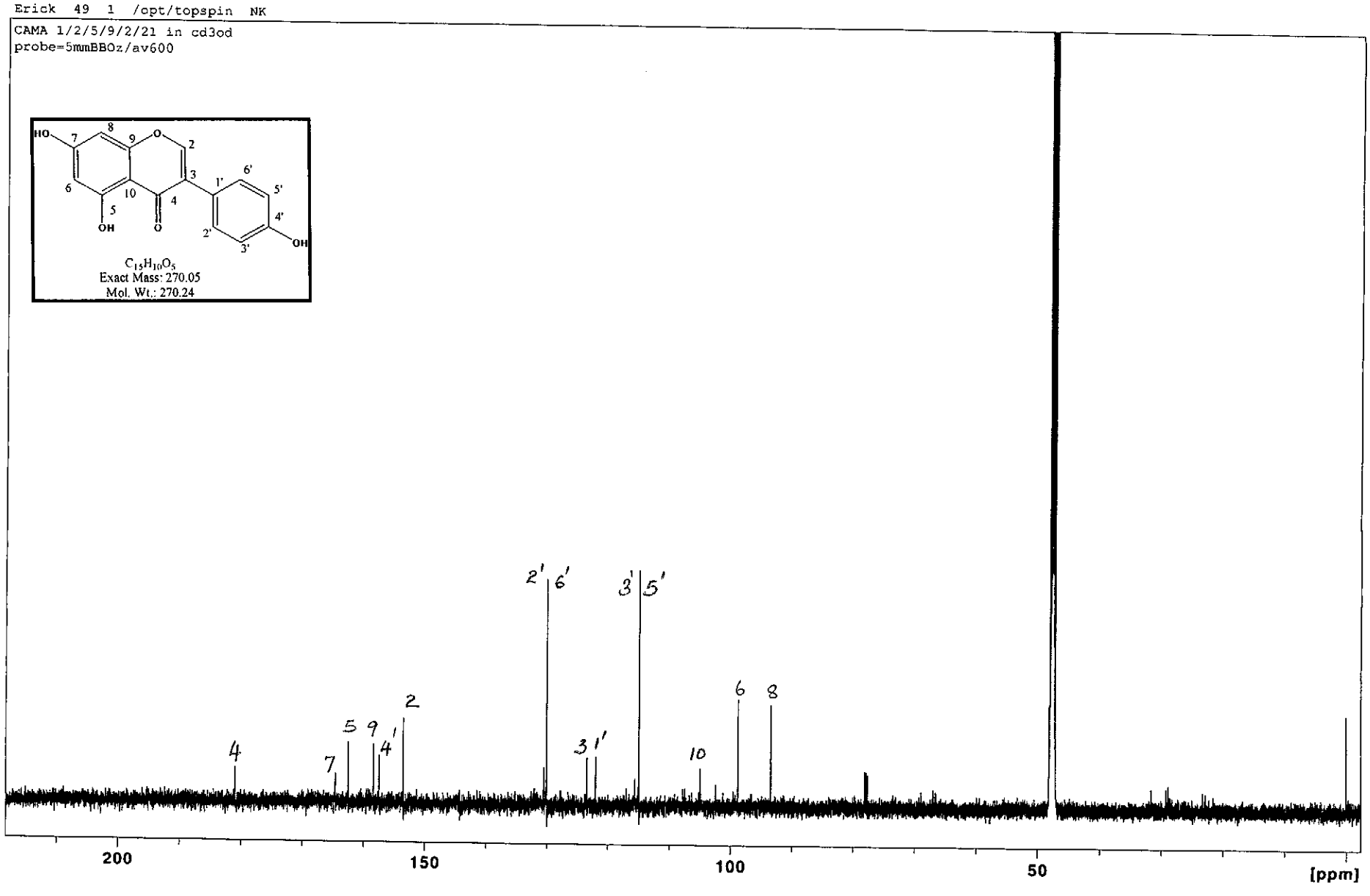


UV spectrum of 12-oleanene-3-one A 10



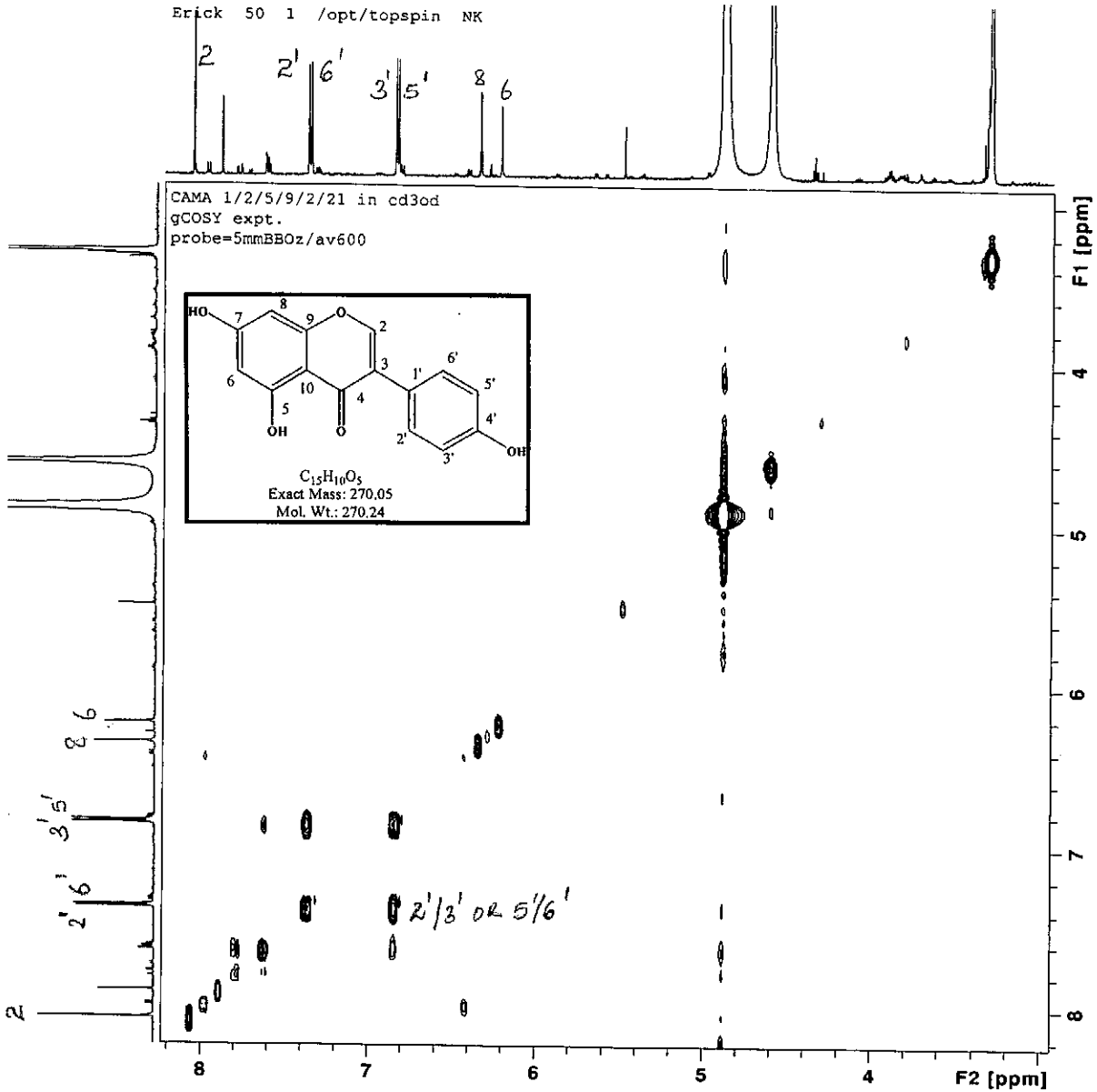
^{1}H NMR spectrum of 4′,5,7-trihydroxyisoflavone B1

Peak	ν(F1) [ppm]	Intensity [abs]	Annotation
1	8.0576	585665.00	
2	7.8880	278020.25	
3	7.3701	198375.75	
4	7.3561	405943.75	
5	6.8466	421608.50	
6	6.8434	126422.00	
7	6.8353	199064.50	
8	6.8319	246023.00	
9	6.3437	302988.50	
10	6.3401	155476.75	
11	6.2196	140571.25	
12	6.2165	205871.75	
13	5.4805	183614.00	

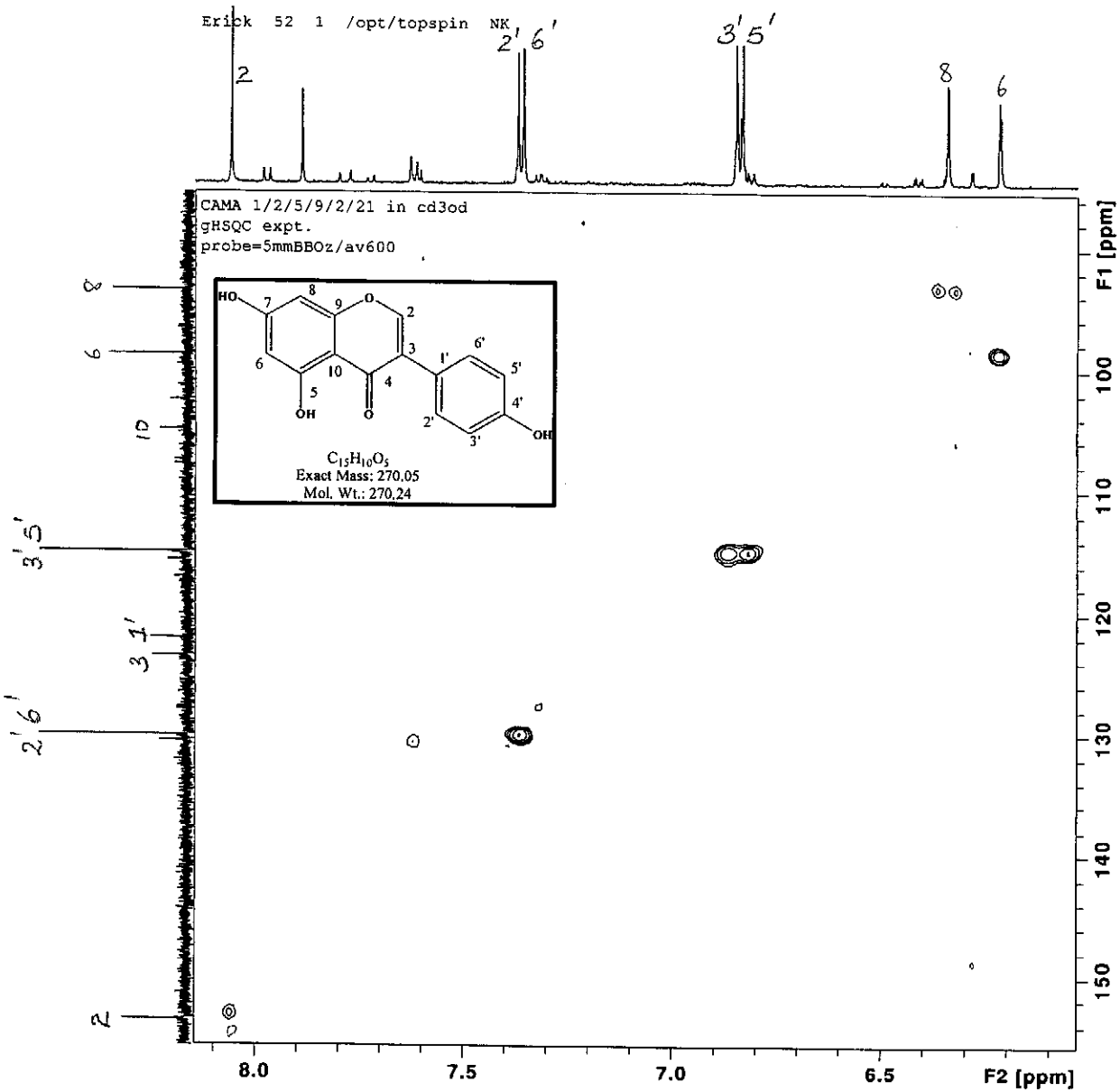


^{13}C NMR spectrum of 4′,5,7-trihydroxyisoflavone B1

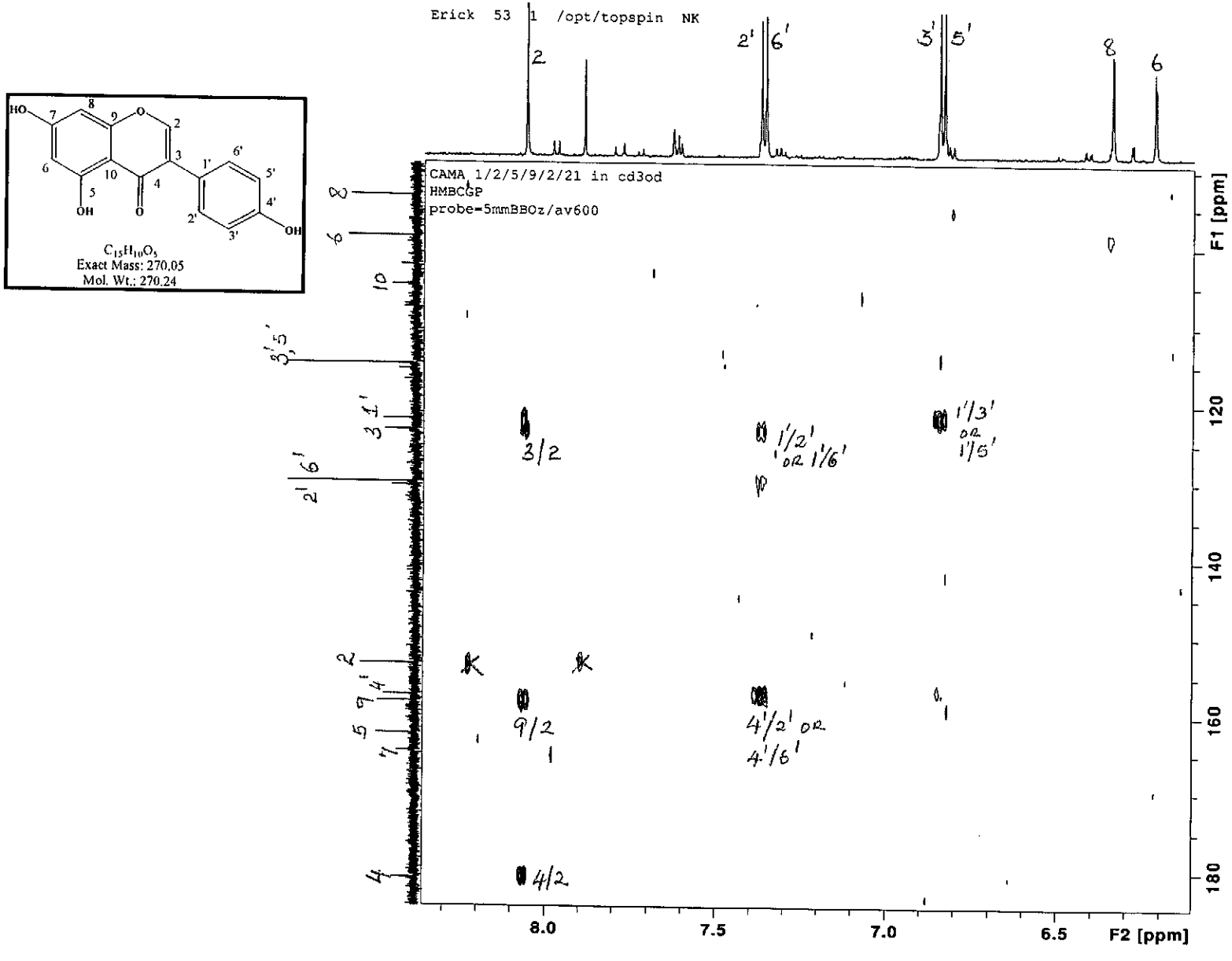
Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity	Annotation
1	180.8813	27294.1317	0.03	
2	164.5720	24833.1356	0.03	
3	162.4496	24512.8754	0.05	
4	158.3367	23892.2583	0.05	
5	157.4038	23751.4881	0.04	
6	153.4379	23153.0526	0.08	
7	130.4438	19683.3518	0.03	
8	129.9889	19614.7095	0.20	
9	123.3486	18612.7197	0.04	
10	121.9100	18395.6418	0.04	
11	115.5316	17433.1714	0.02	
12	114.8735	17333.8672	0.21	
13	104.8888	15827.2233	0.03	
14	102.4089	15453.0181	0.02	
15	98.7284	14897.6481	0.10	
16	93.3952	14092.8935	0.09	
17	78.0484	11777.1340	0.03	
18	77.8633	11749.2033	0.03	
19	77.6454	11716.3252	0.03	
20	48.1579	7266.7991	0.10	
21	48.0162	7245.4172	2.55	
22	47.8754	7224.1712	6.36	
23	47.7320	7202.5328	14.22	
24	47.4479	7159.6634	13.72	
25	47.3076	7138.4928	6.66	
26	47.1638	7116.7941	2.22	
27	31.6665	4778.3249	0.02	
28	29.2821	4418.5302	0.02	
29	28.8827	4359.0171	0.02	
30	0.0617	9.3102	0.09	



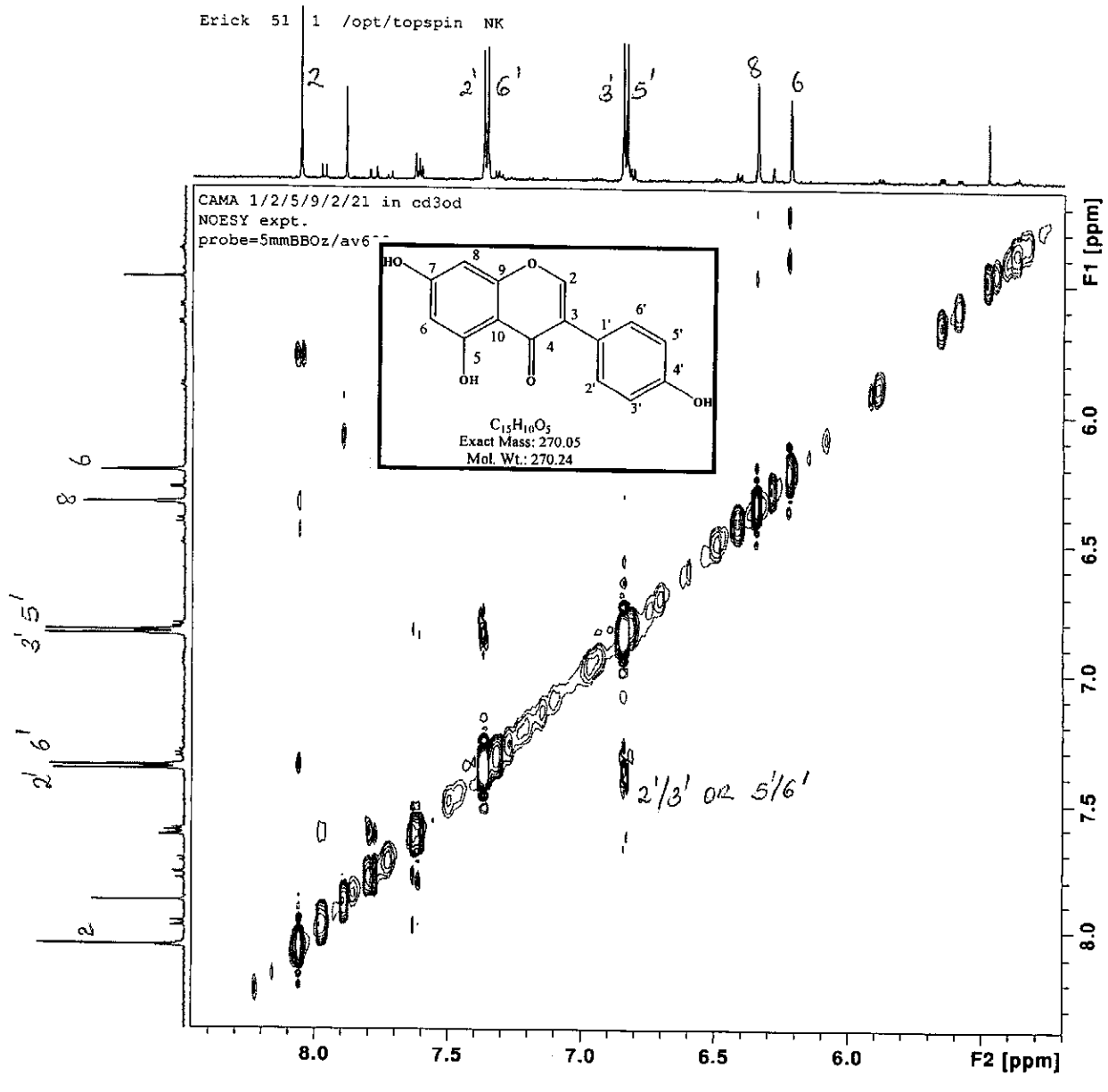
COSY spectrum of 4′,5,7-trihydroxyisoflavone 61



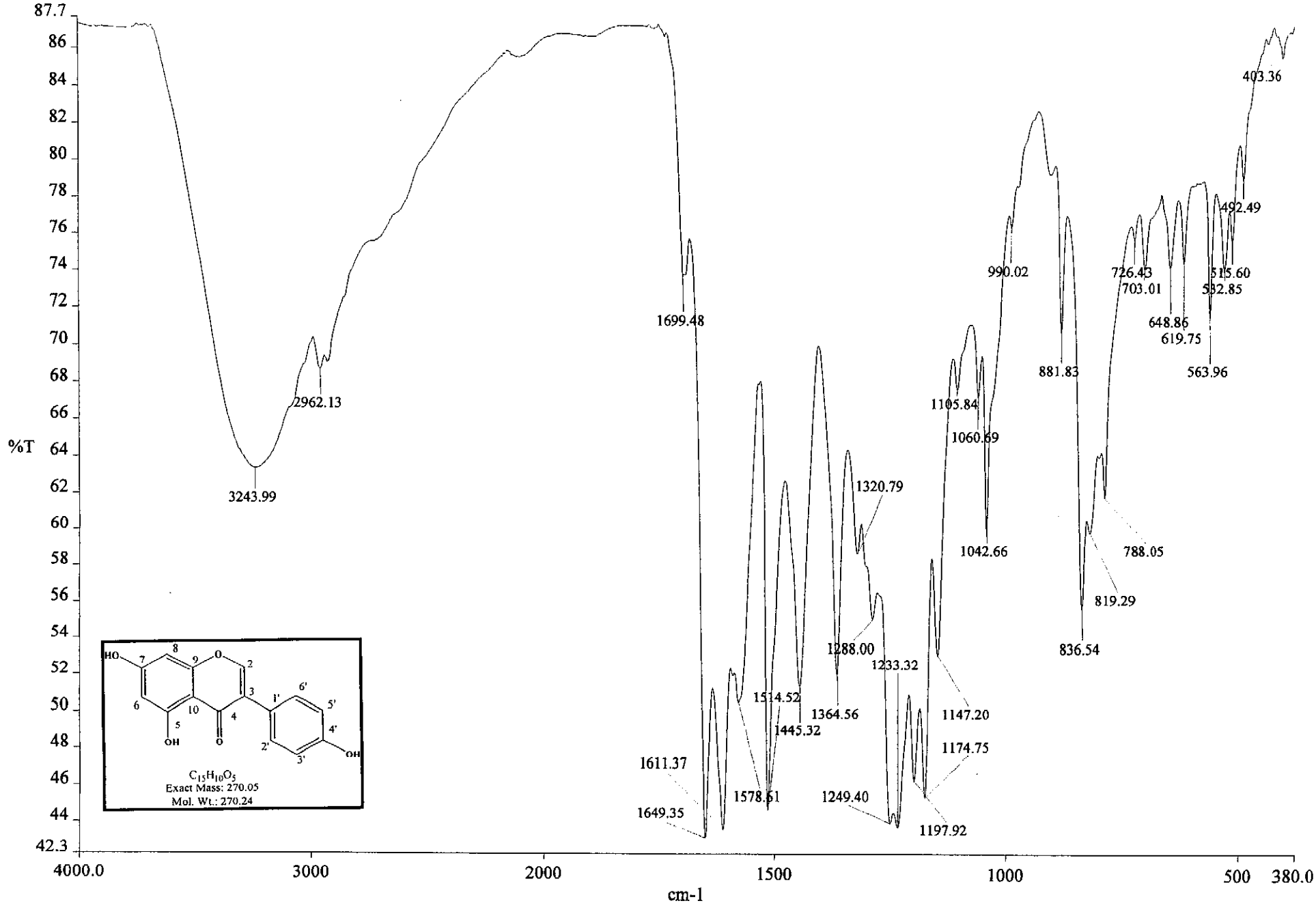
HSQC spectrum of 4′,5,7-trihydroxyisoflavone B1



HMBC spectrum of 4′,5,7-trihydroxyisoflavone B1

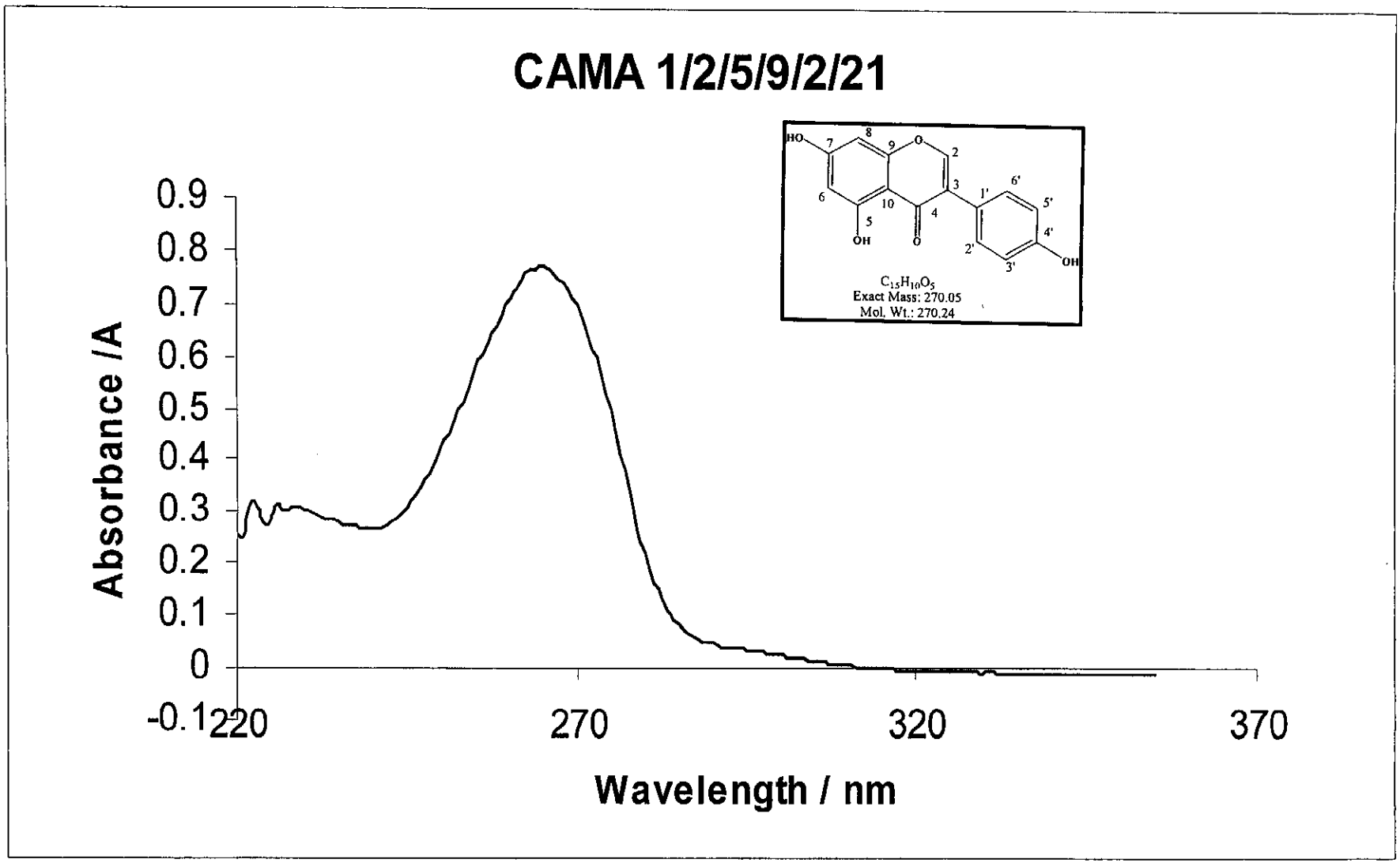


NOESY spectrum of 4′,5,7-trihydroxyisoflavone 81



c:\pel_data\spectra\cama 1-2-5 9-2-21.002

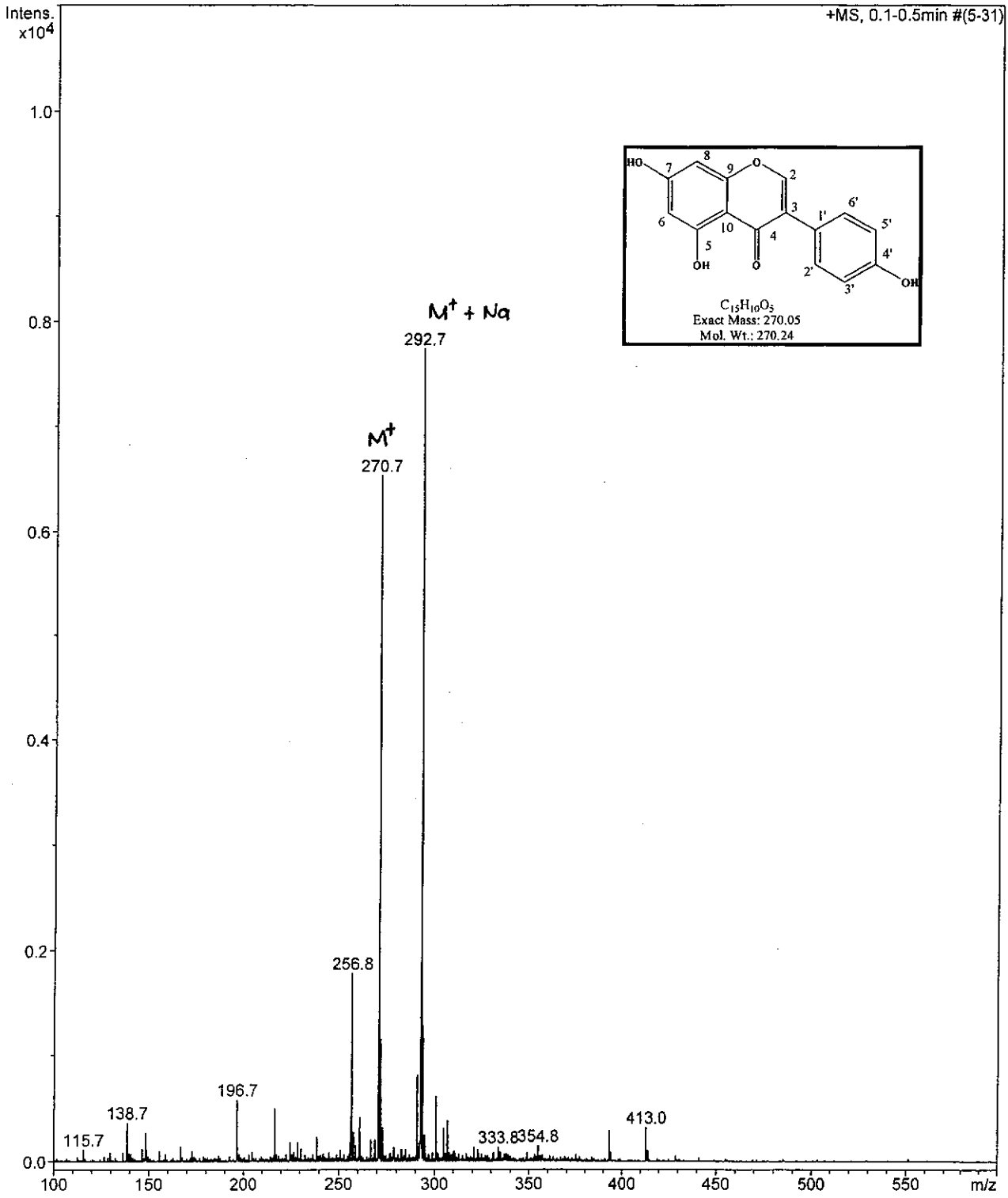
IR spectrum of 4′,5,7-trihydroxyisoflavone B1



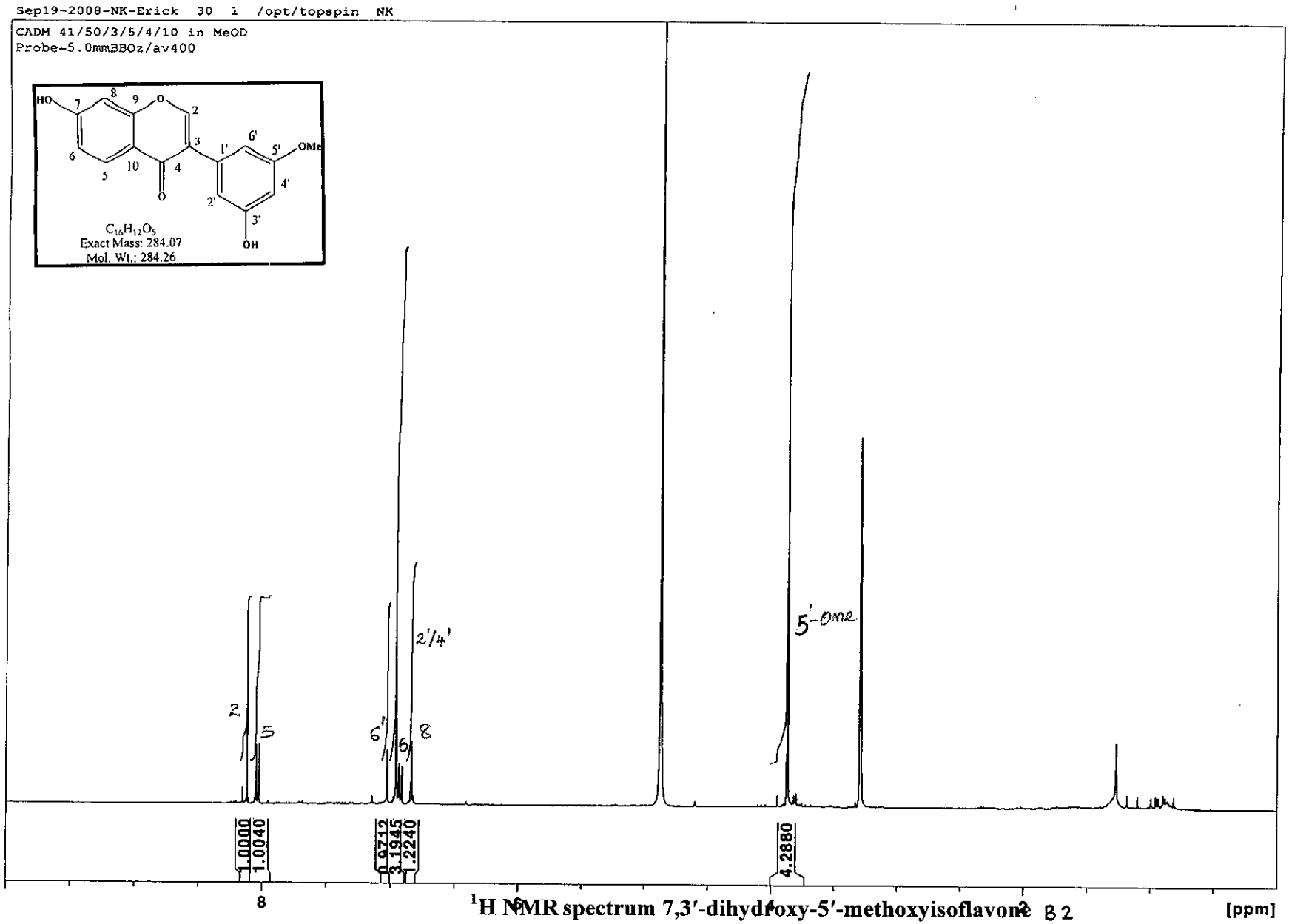
UV spectrum of 4′,5,7-trihydroxyisoflavone B1

Display Report - All Windows Selected Analysis

Analysis Name: ERICK1.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 10/12/2009 08:30:57 PM
Method: FIA.M **Operator:** Administrator **Acq. Date:** 10/12/2009 7:43:17 PM
Sample Name: ERICK1
Analysis Info: SOLUBLE IN METHANOL

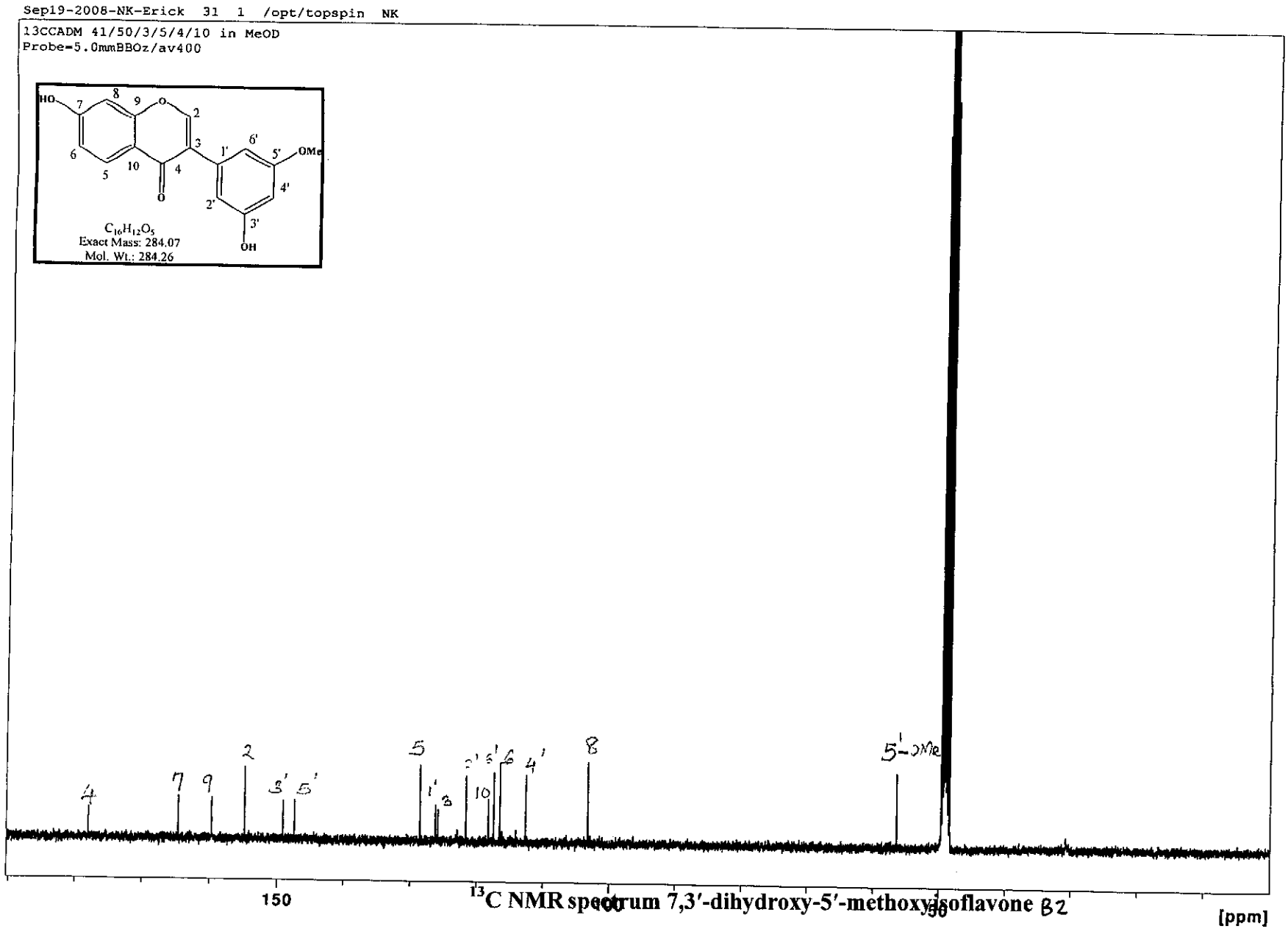


Mass spectrum of 4,5,7-trihydroxyisoflavone B1



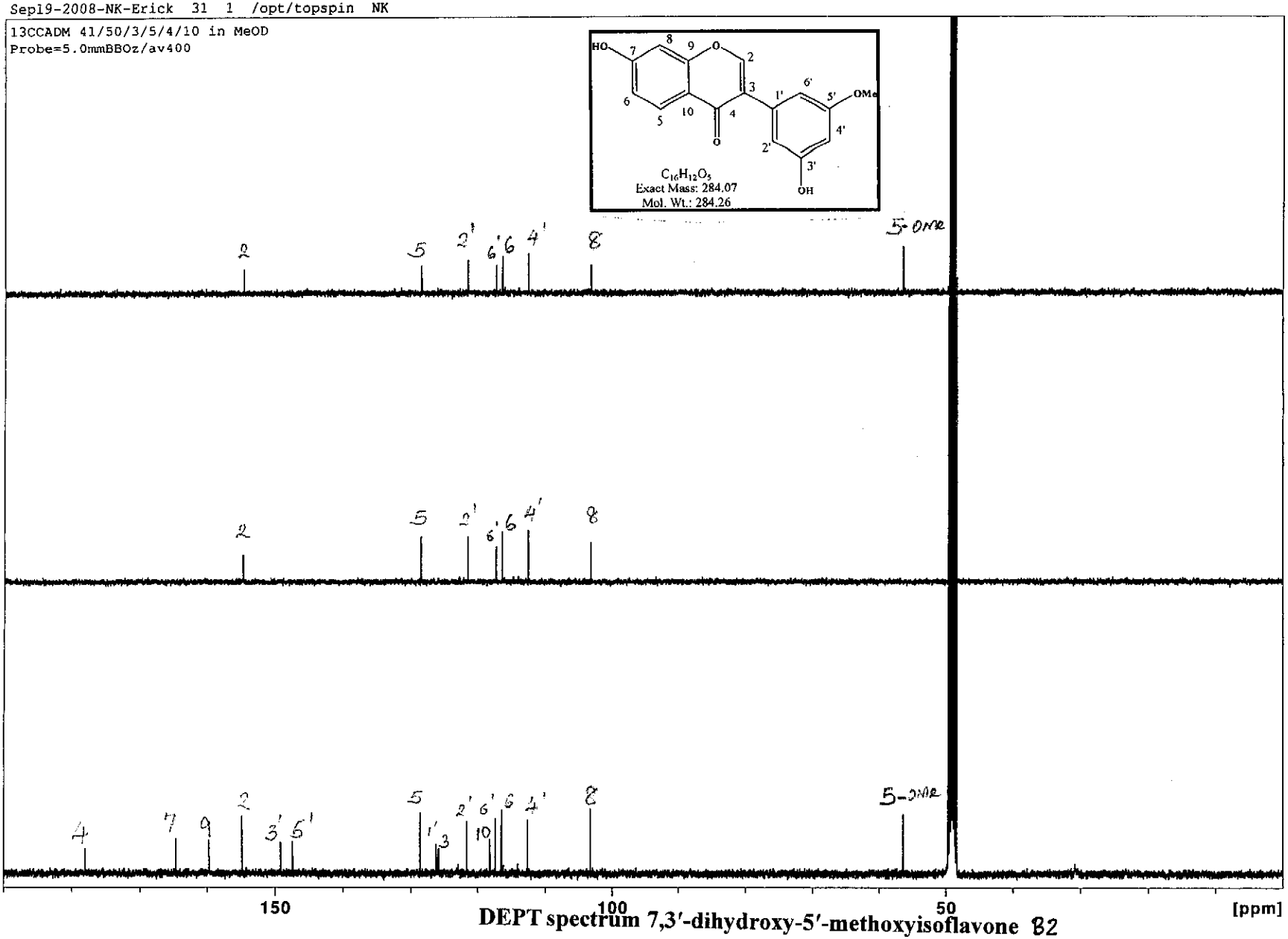
^{1}H NMR spectrum 7,3′-dihydroxy-5′-methoxyisoflavone B 2

Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity [rel]	Annotation
2	8.1027	3242.8628	2.55	
4	8.0324	3214.7273	1.14	
6	8.0102	3205.8424	1.15	
8	7.1247	2851.4476	0.16	
10	7.0075	2804.5418	1.06	
12	6.9400	2777.5269	2.79	
14	6.9150	2767.5214	0.78	
16	6.8985	2760.9178	0.59	
18	6.8270	2732.3021	0.44	
20	6.8161	2727.9397	1.10	
22	3.9313	1573.3850	0.23	
24	3.8488	1540.3668	7.45	
26	3.7826	1513.8722	0.26	
28	3.2794	1312.4815	4.83	
30	3.2717	1309.3998	4.97	
32	1.2521	501.1155	1.26	
34	1.0844	433.9986	0.23	
36	0.9398	376.1268	0.24	
38	0.8809	352.5538	0.28	
40	0.8485	339.5867	0.16	

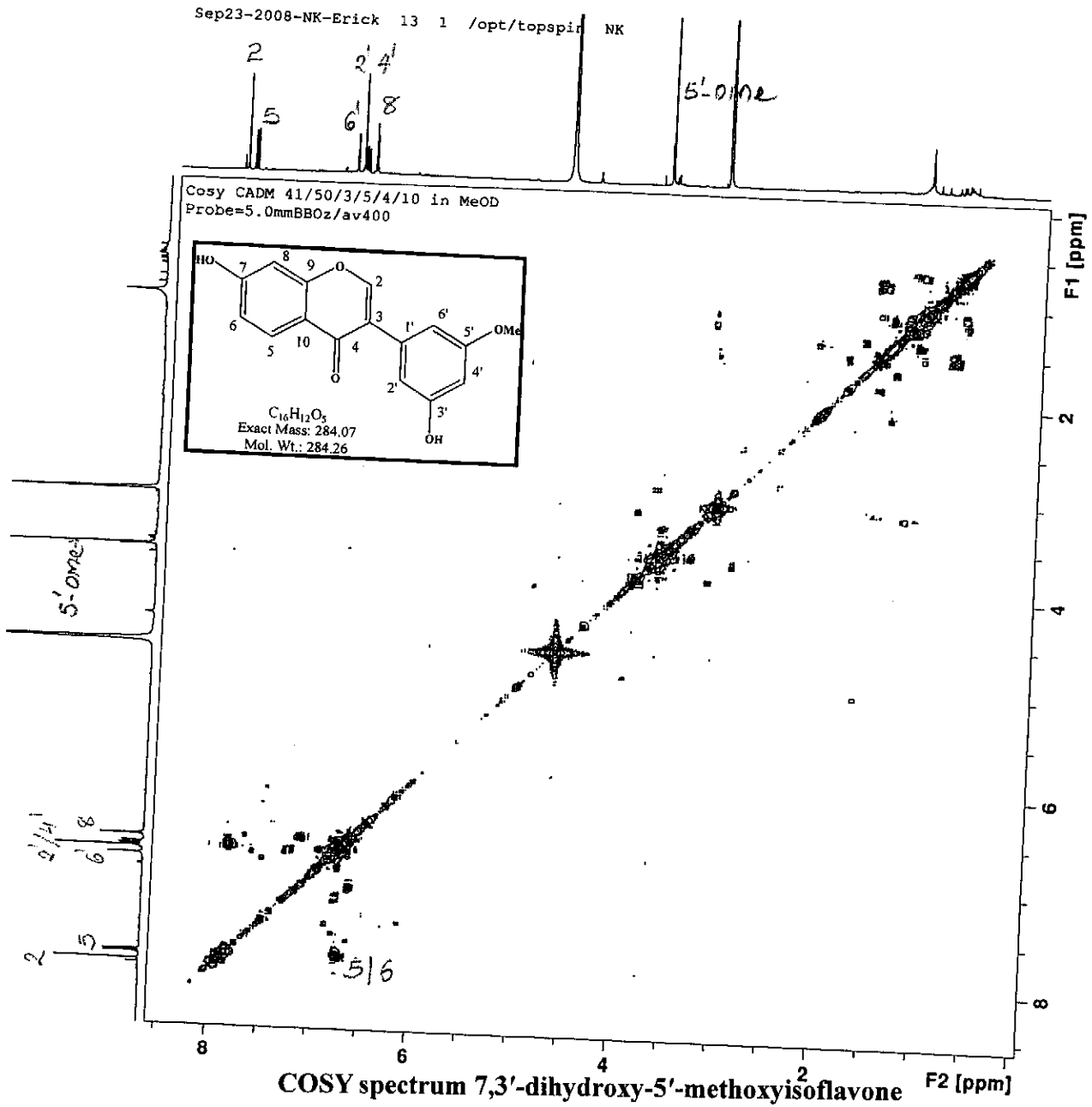


^{13}C NMR spectrum 7,3′-dihydroxy-5′-methoxyisoflavone B2

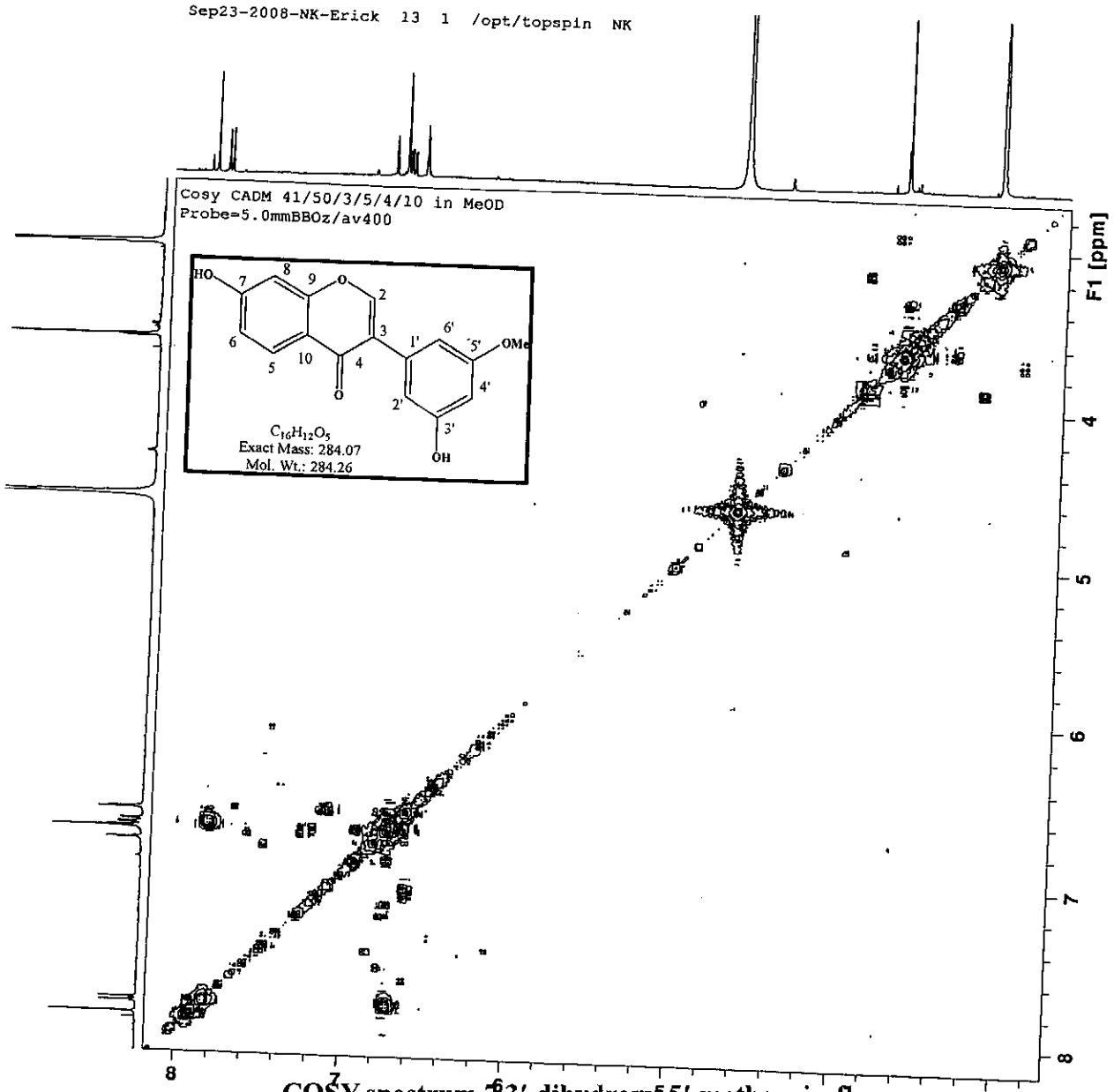
Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity [rel]	Annotation
2	164.6620	16570.8025	0.11	
4	154.8792	15586.3080	0.18	
6	147.4361	14837.2697	0.10	
8	126.2008	12700.2498	0.09	
10	122.8822	12366.2817	0.03	
12	118.2042	11895.5099	0.11	
14	116.4769	11721.6827	0.20	
16	114.0705	11479.5140	0.03	
18	103.2472	10390.3084	0.20	
20	49.5073	4982.1798	0.17	
22	30.8062	3100.1898	0.03	



DEPT spectrum 7,3′-dihydroxy-5′-methoxyisoflavone B2

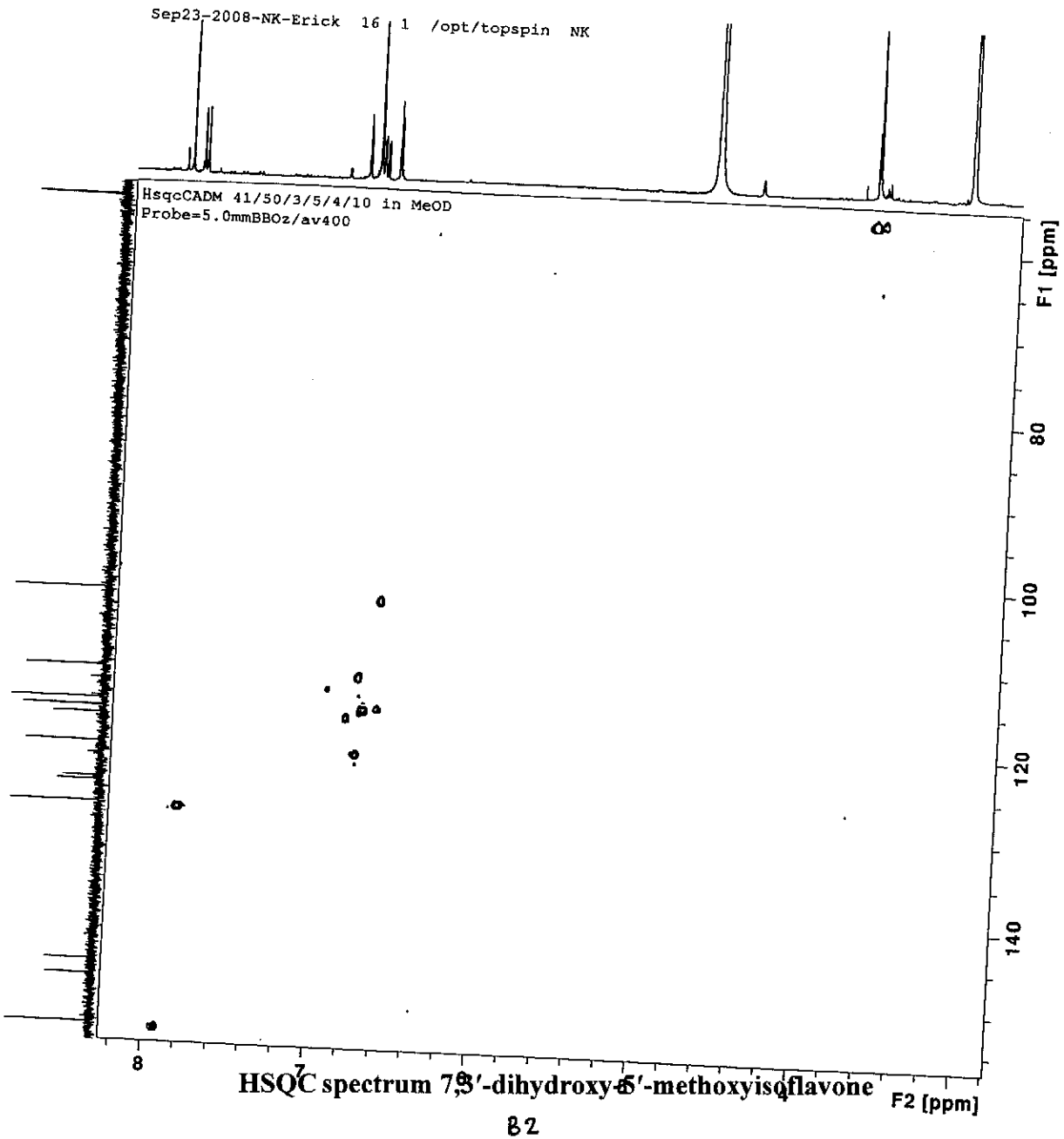


COSY spectrum 7,3′-dihydroxy-5′-methoxyisoflavone

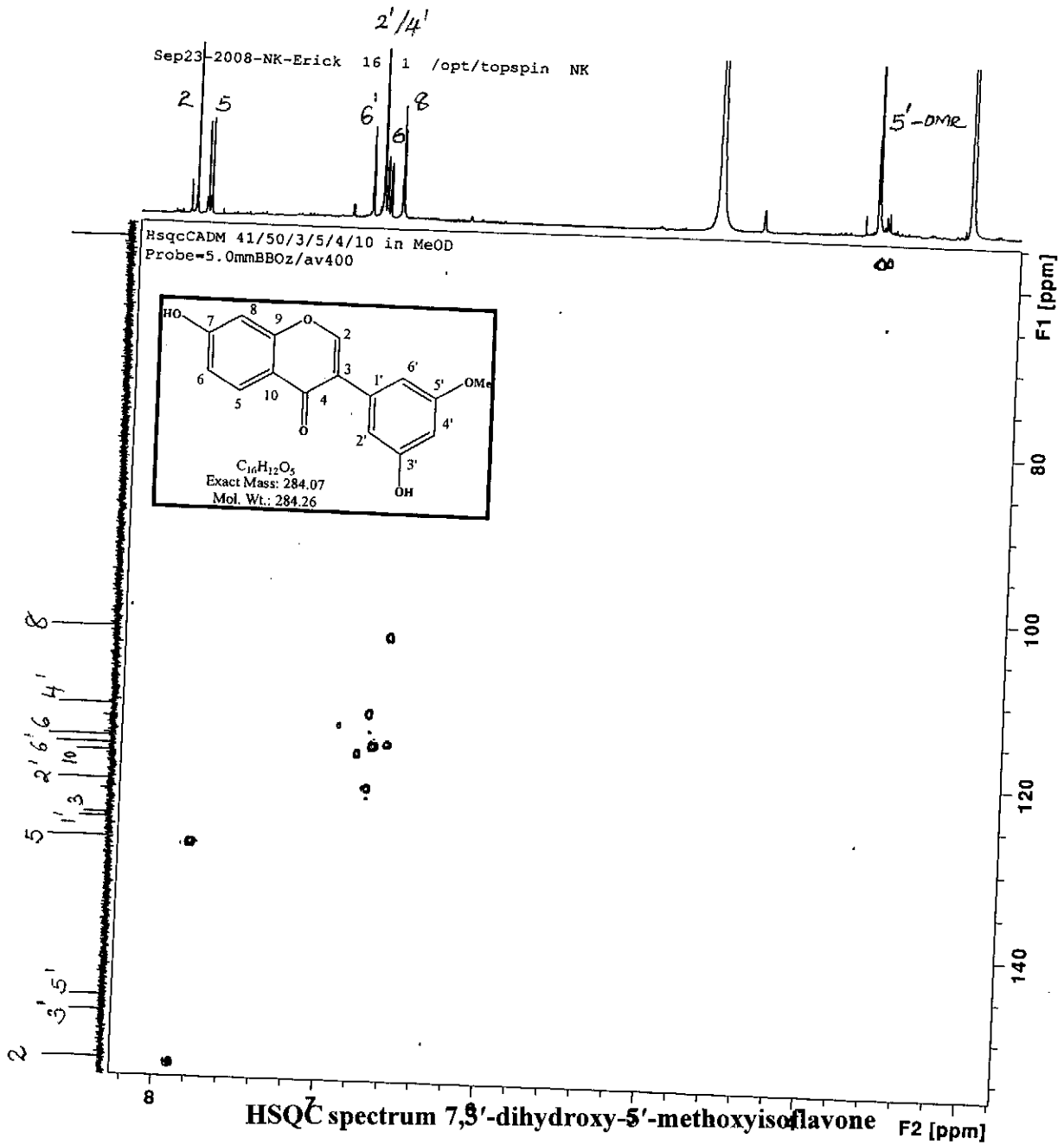


COSY spectrum 7,3′-dihydroxy-5′-methoxyisoflavone

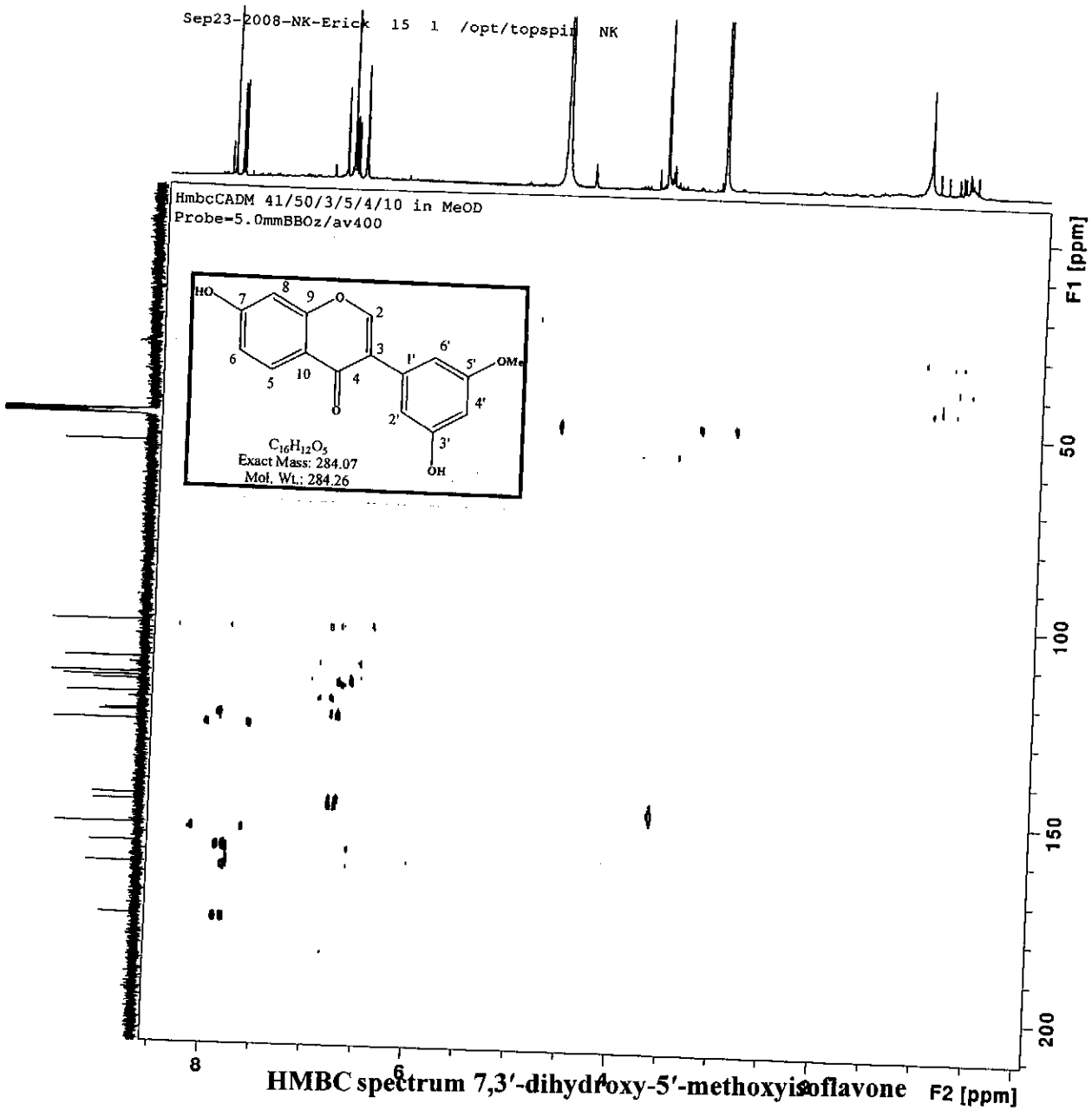
B2



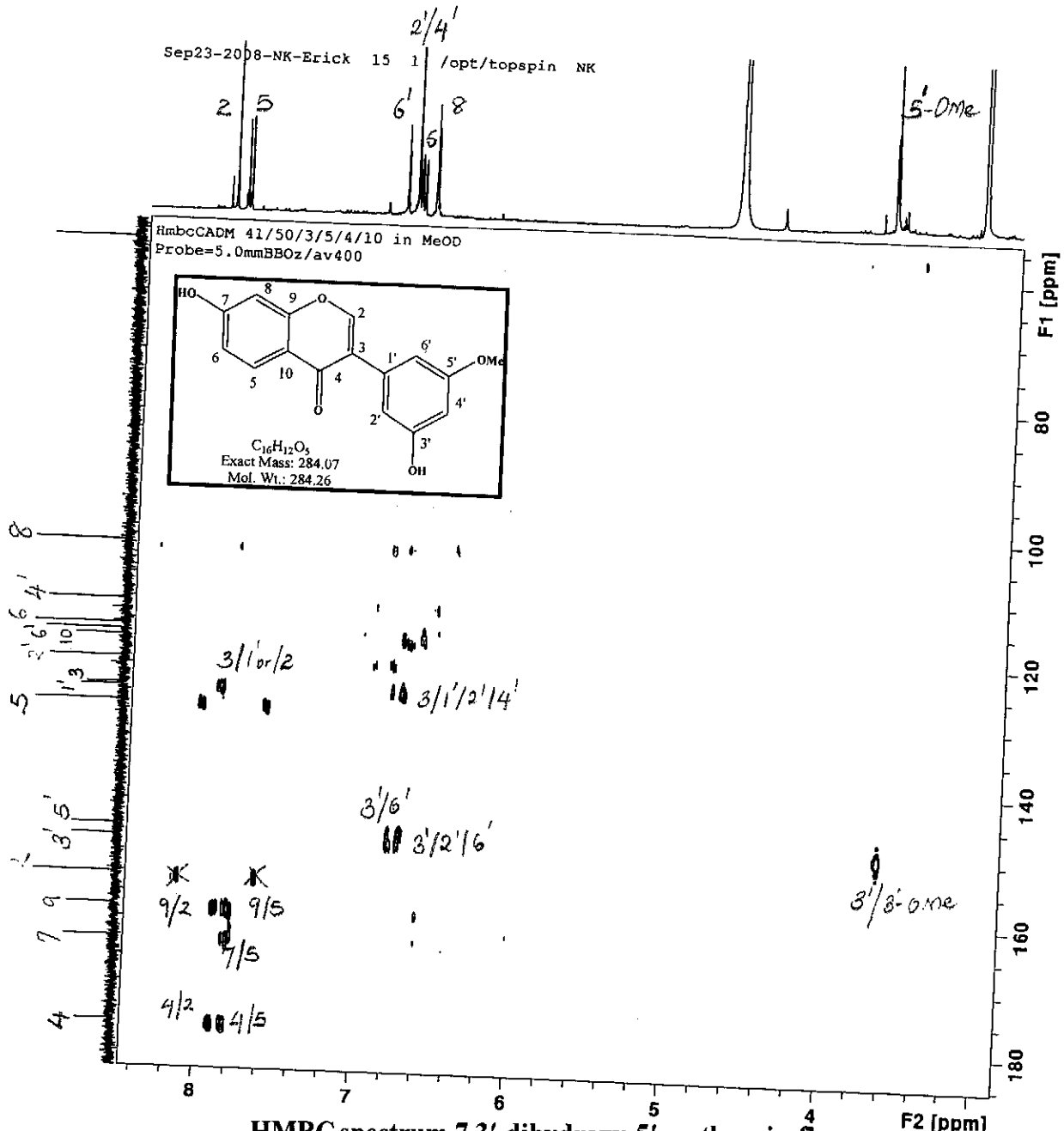
HSQC spectrum 7,3′-dihydroxy-5′-methoxyisoflavone



HSQC spectrum 7,3′-dihydroxy-5′-methoxyisoflavone

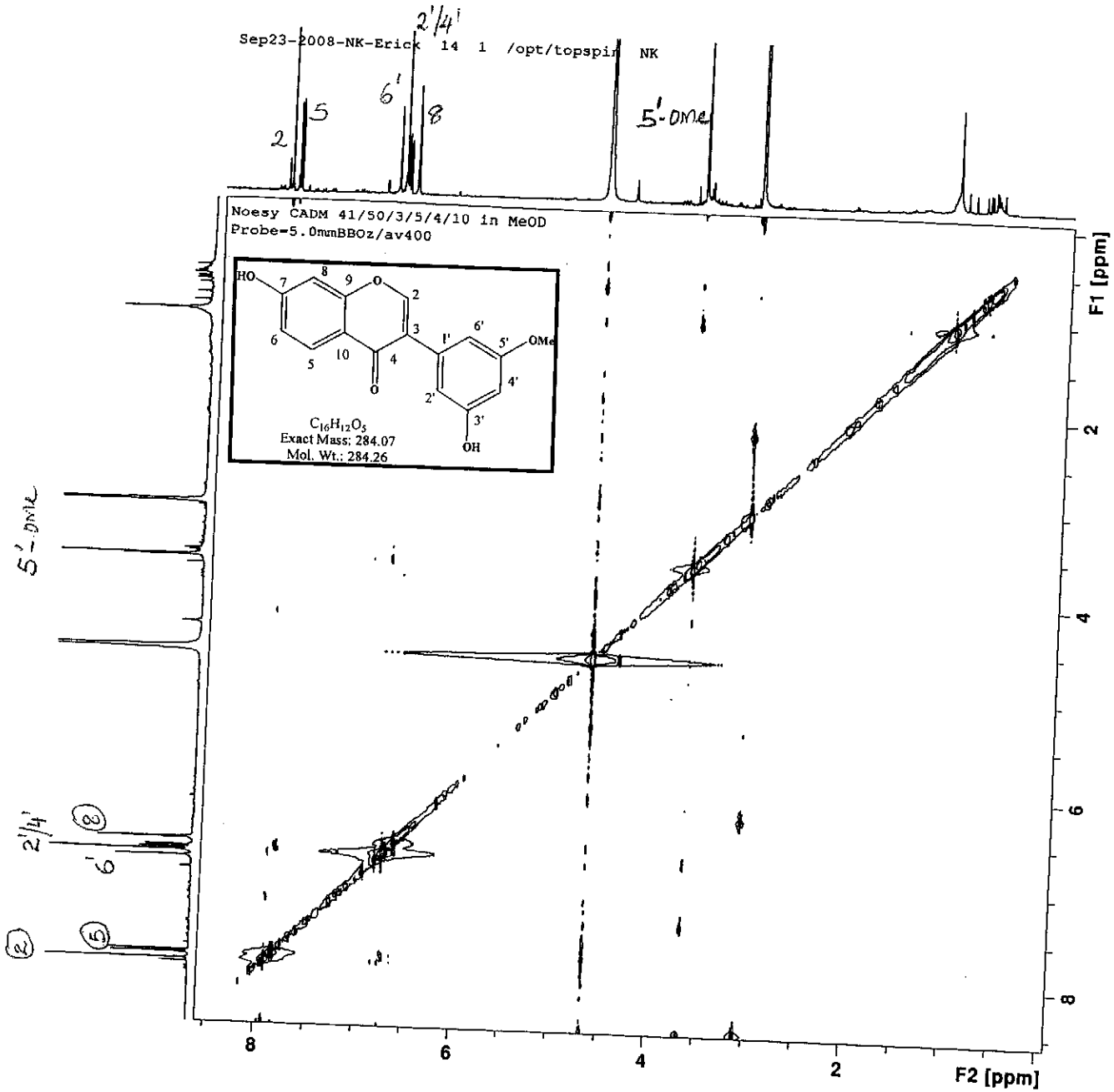


HMBC spectrum 7,3′-dihydroxy-5′-methoxyisoflavone

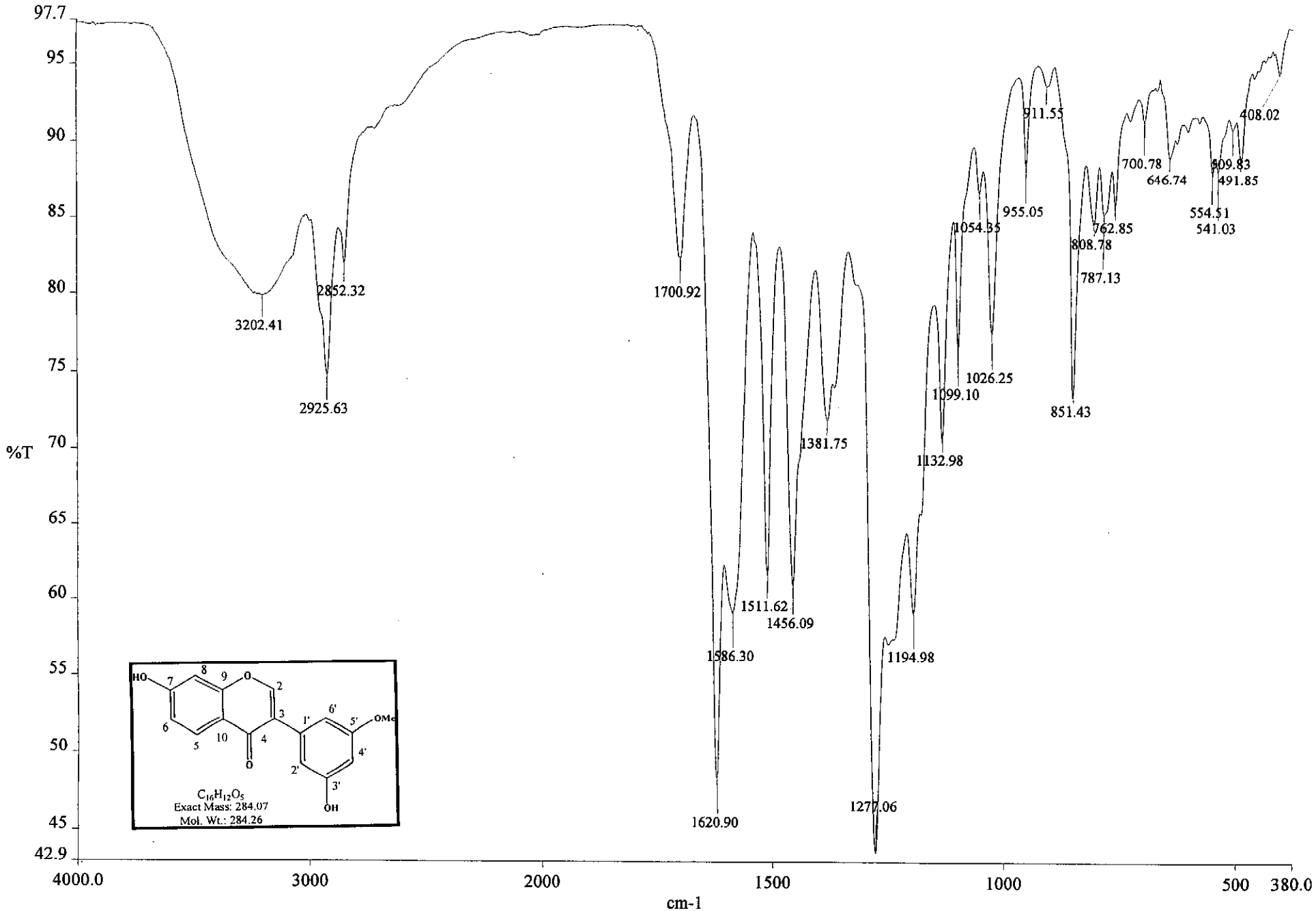


HMBC spectrum 7,3′-dihydroxy-5′-methoxyisoflavone

B2

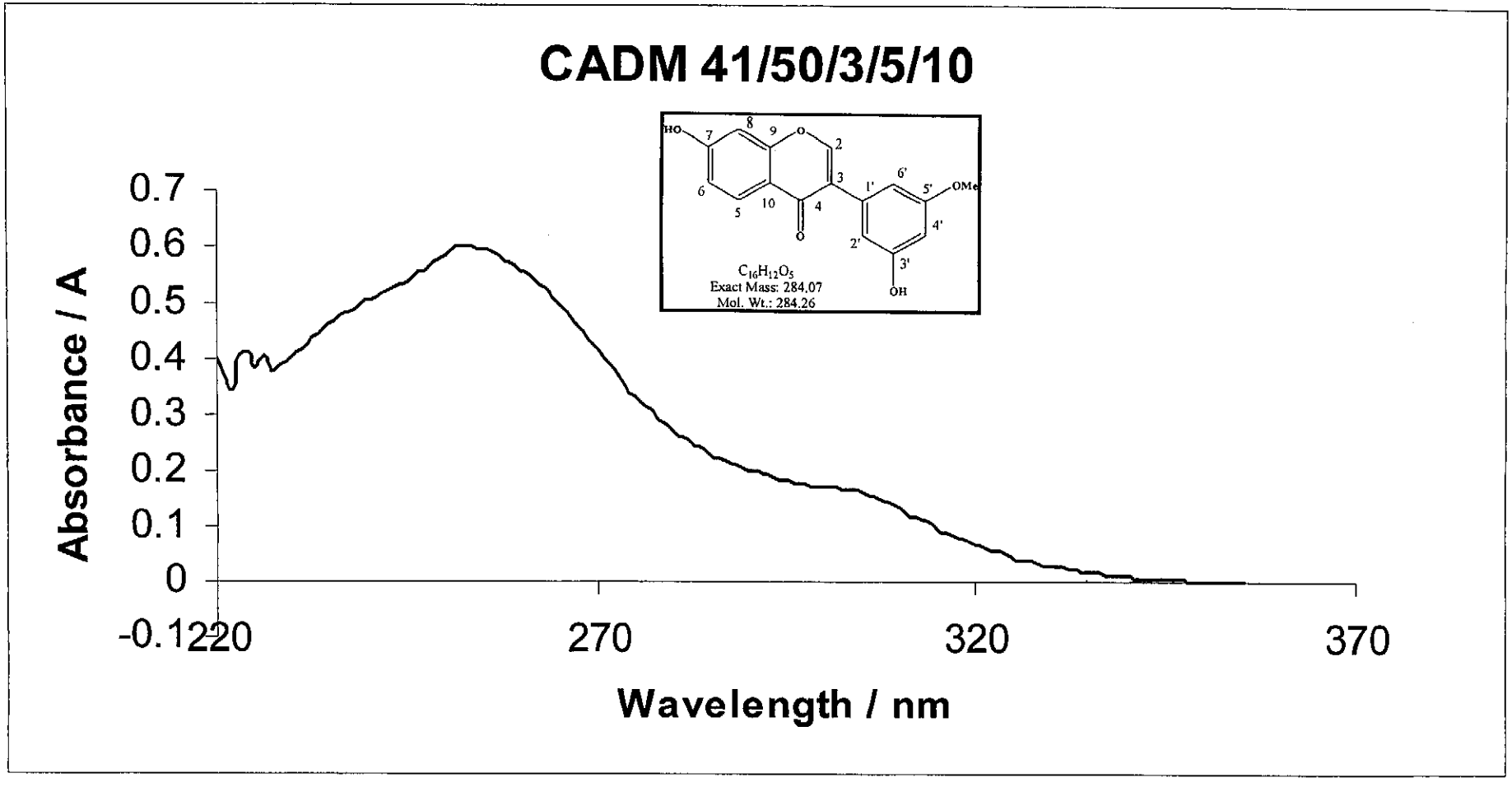


NOESY spectrum 7,3′-dihydroxy-5′-methoxyisoflavone B2



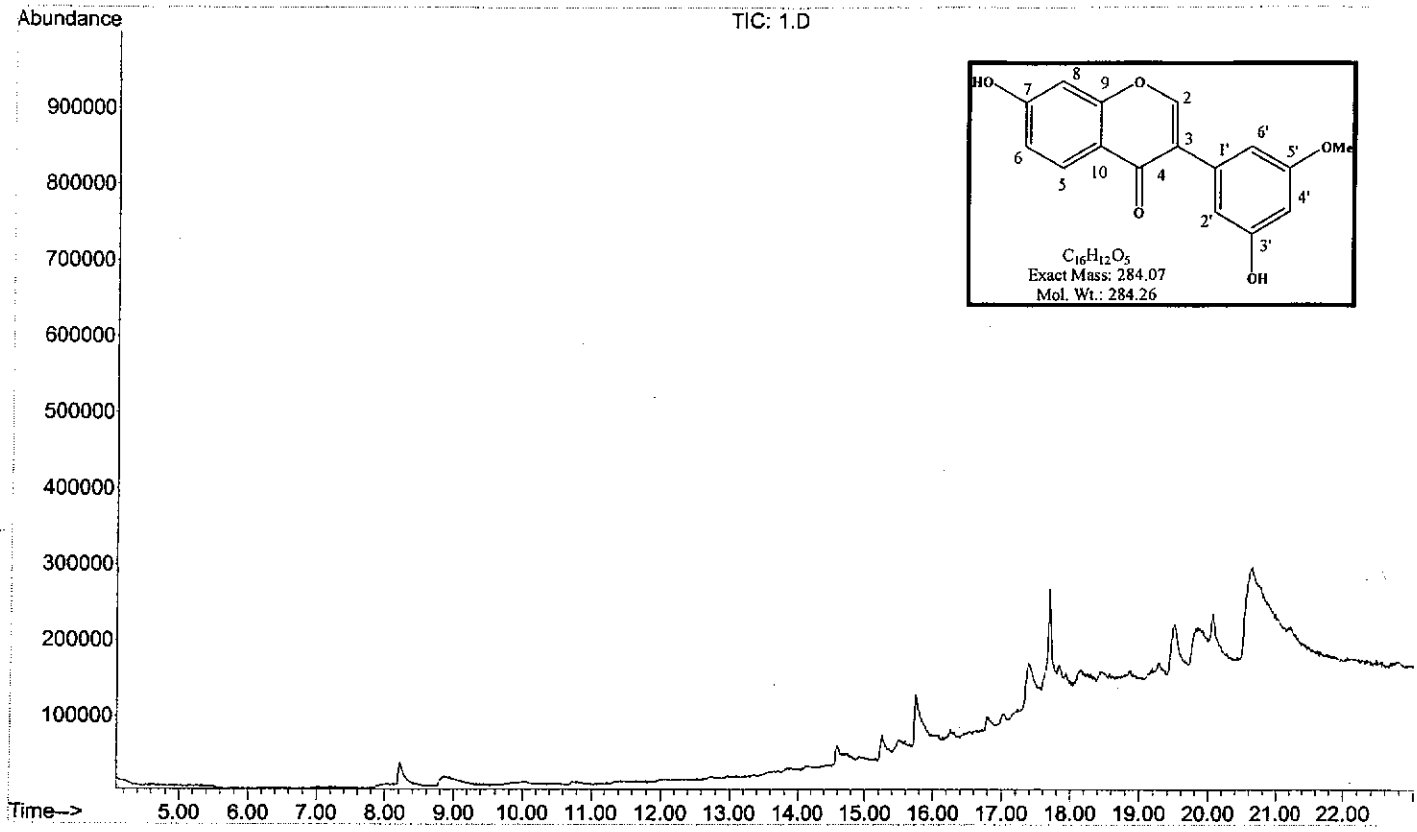
c:\pel_data\spectra\cadm 41-50-3-5-4-10.002

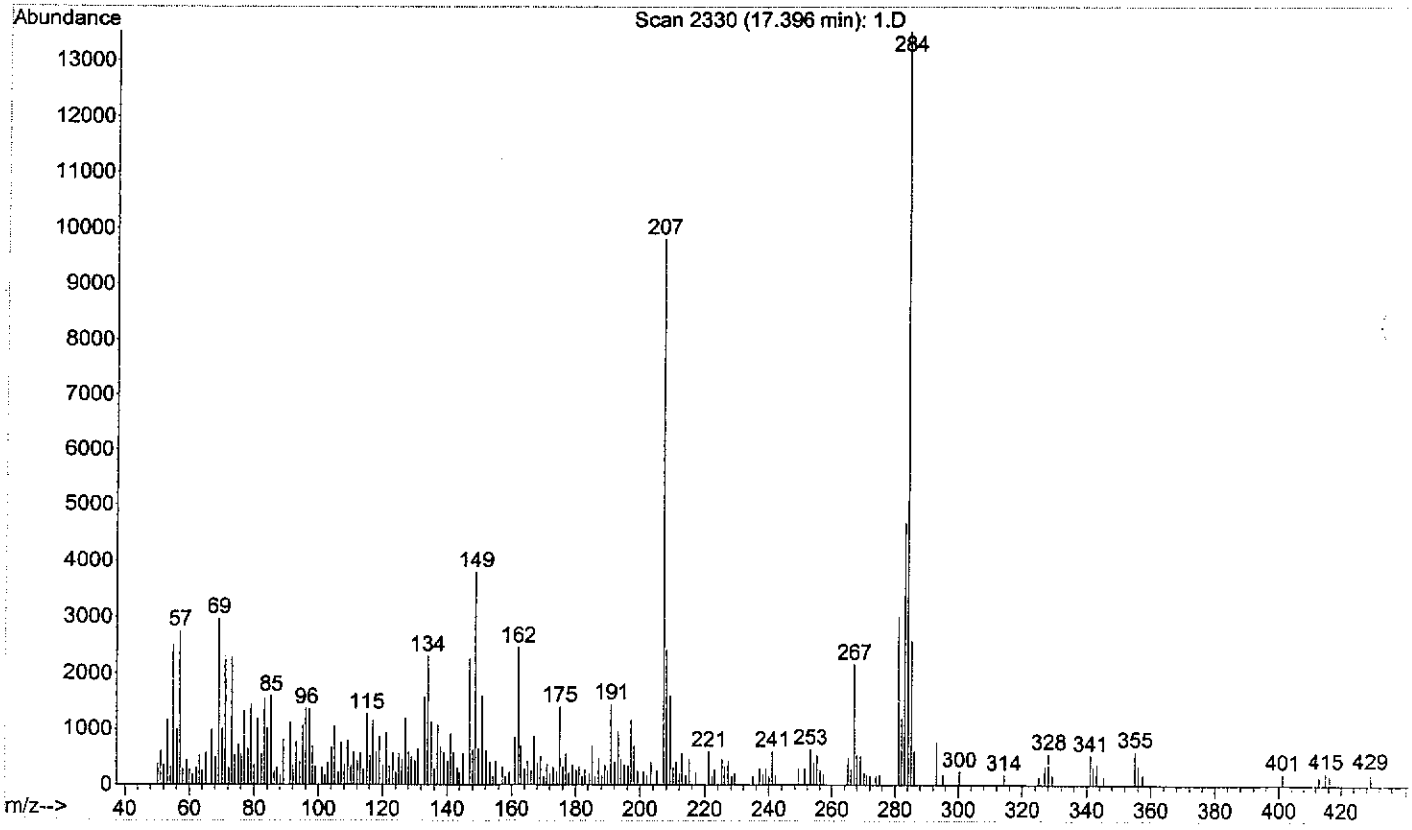
IR spectrum 7,3′-dihydroxy-5′-methoxyisoflavone B2



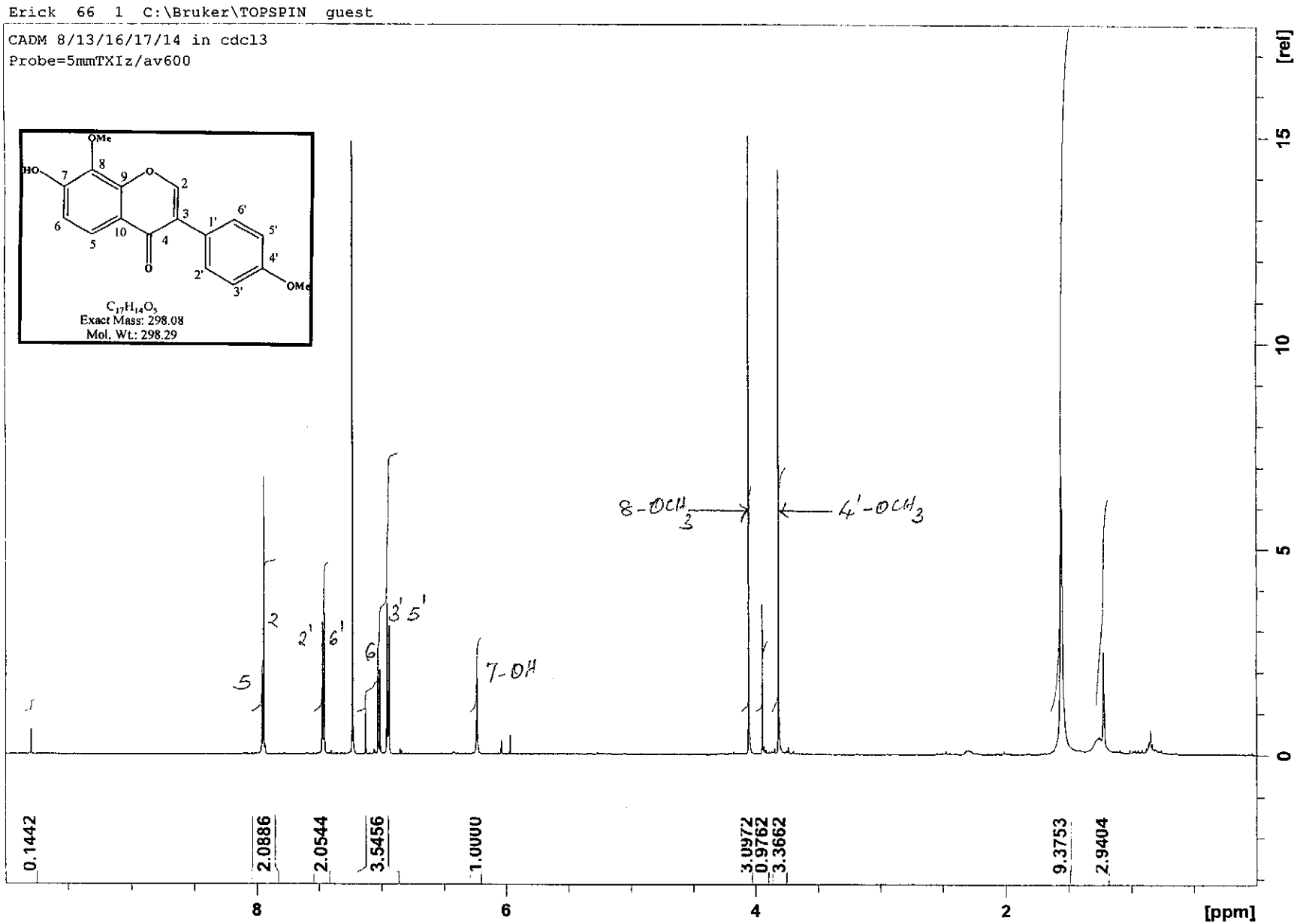
UV spectrum 7,3′-dihydroxy-5′-methoxyisoflavone 82

```
File       : D:\ERICK2009\1.D
Operator   : ERICK
Acquired   :  4 Sep 2009  19:19     using AcqMethod ERICK1
Instrument :    Instrumen
Sample Name: 040909 RERUN 3ULCHANGED OVEN TEMP
Misc Info  :
Vial Number: 1
```



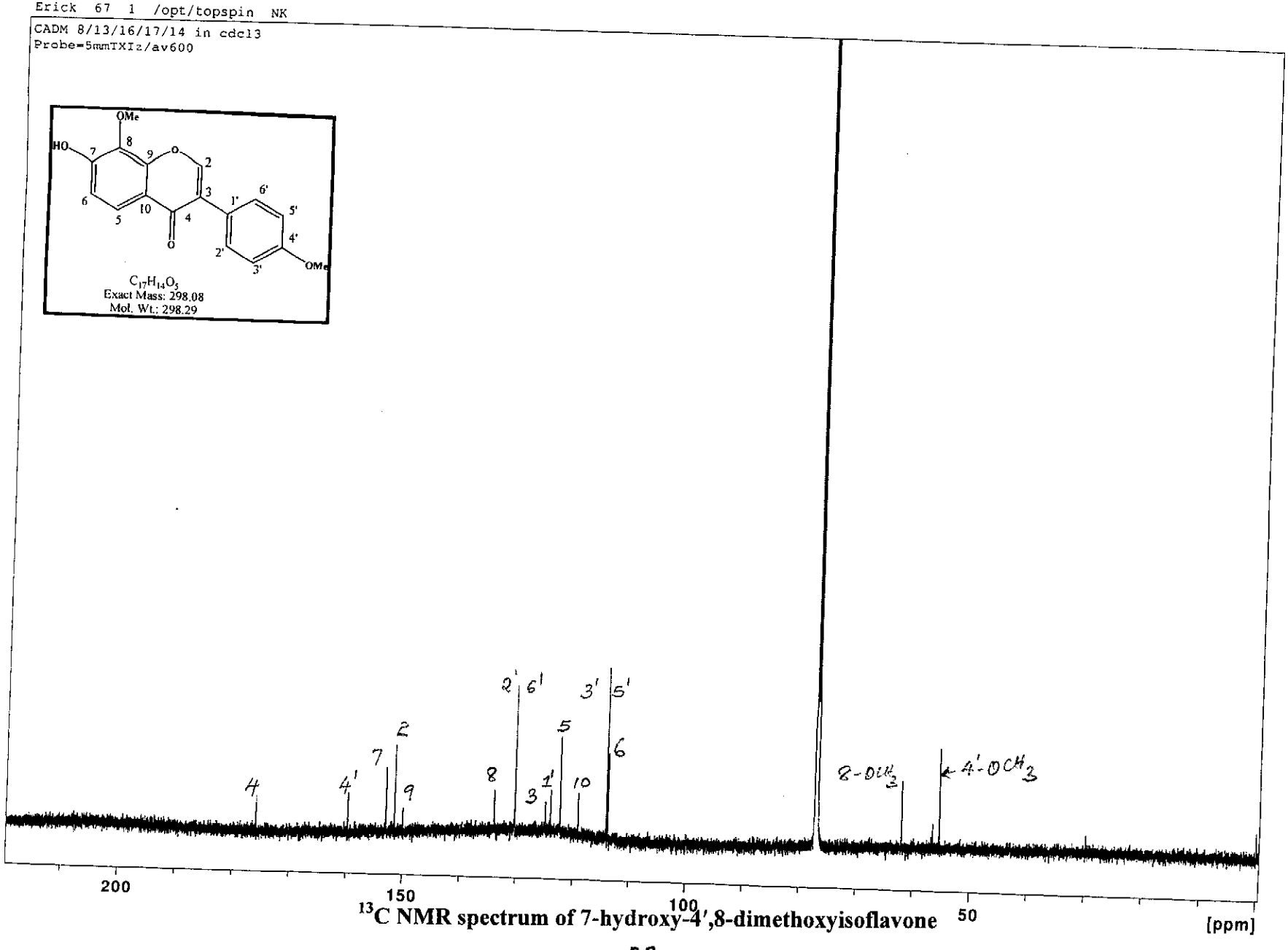


Mass spectrum of 7,3′-dihydroxy-5′-methoxyisoflavone B2



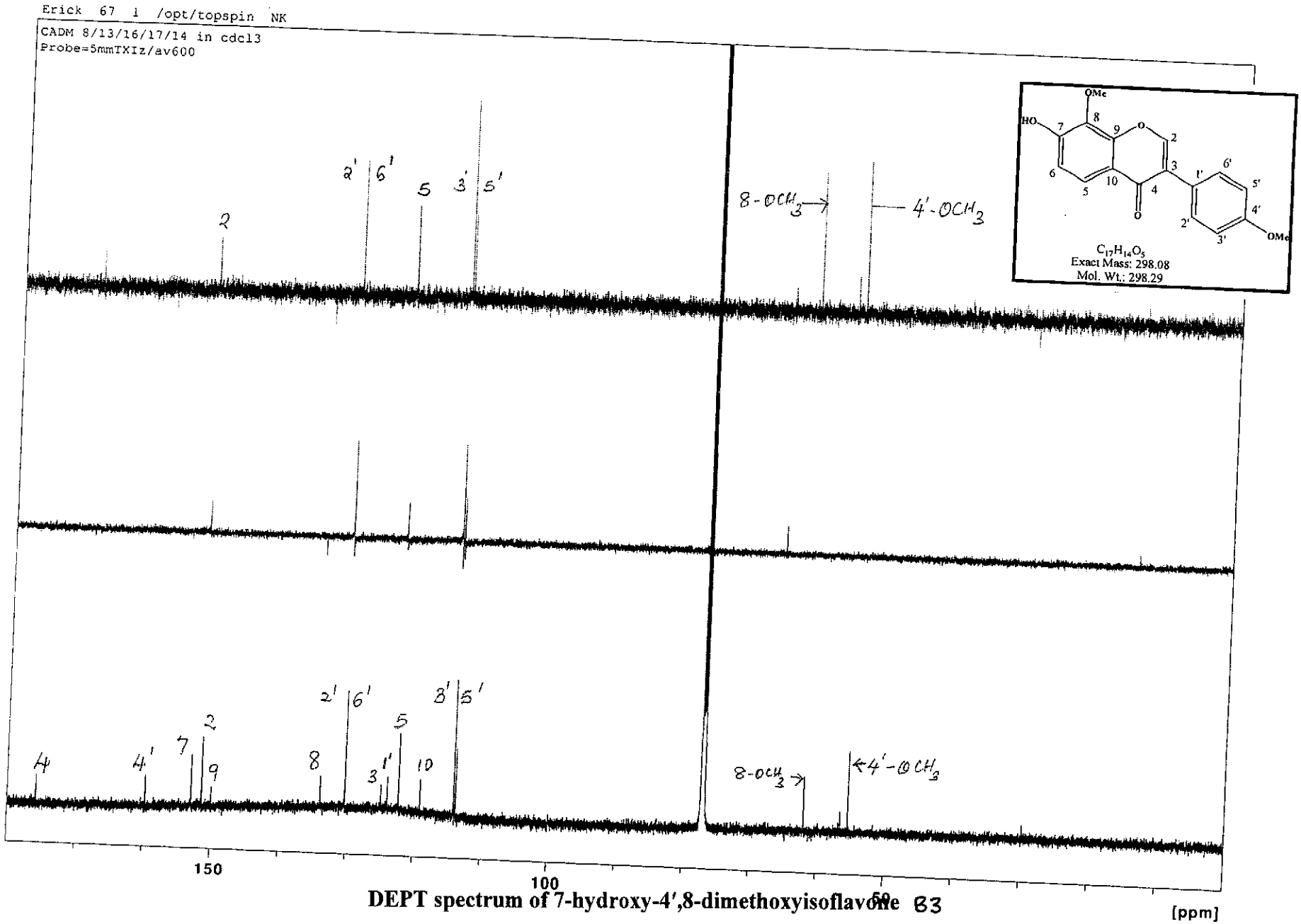
^{1}H NMR spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone B3

Peak	ν(F1) [ppm]	Intensity [abs]	Annotation
1	9.7905	484793.88	
2	7.9552	2225951.69	
3	7.9411	5285812.12	
4	7.4709	3292527.62	
5	7.4567	4128156.50	
6	7.2289	16265475.00	
7	7.1244	880213.75	
8	7.0254	2178256.38	
9	7.0108	2439981.75	
10	6.9536	4392726.44	
11	6.9390	3436980.75	
12	6.2349	2048599.25	
13	4.0530	10851085.81	
14	3.9453	3208444.94	
15	3.8144	17001284.75	
16	1.5556	8164393.56	
17	1.2193	2969311.81	
18	0.8461	700037.81	

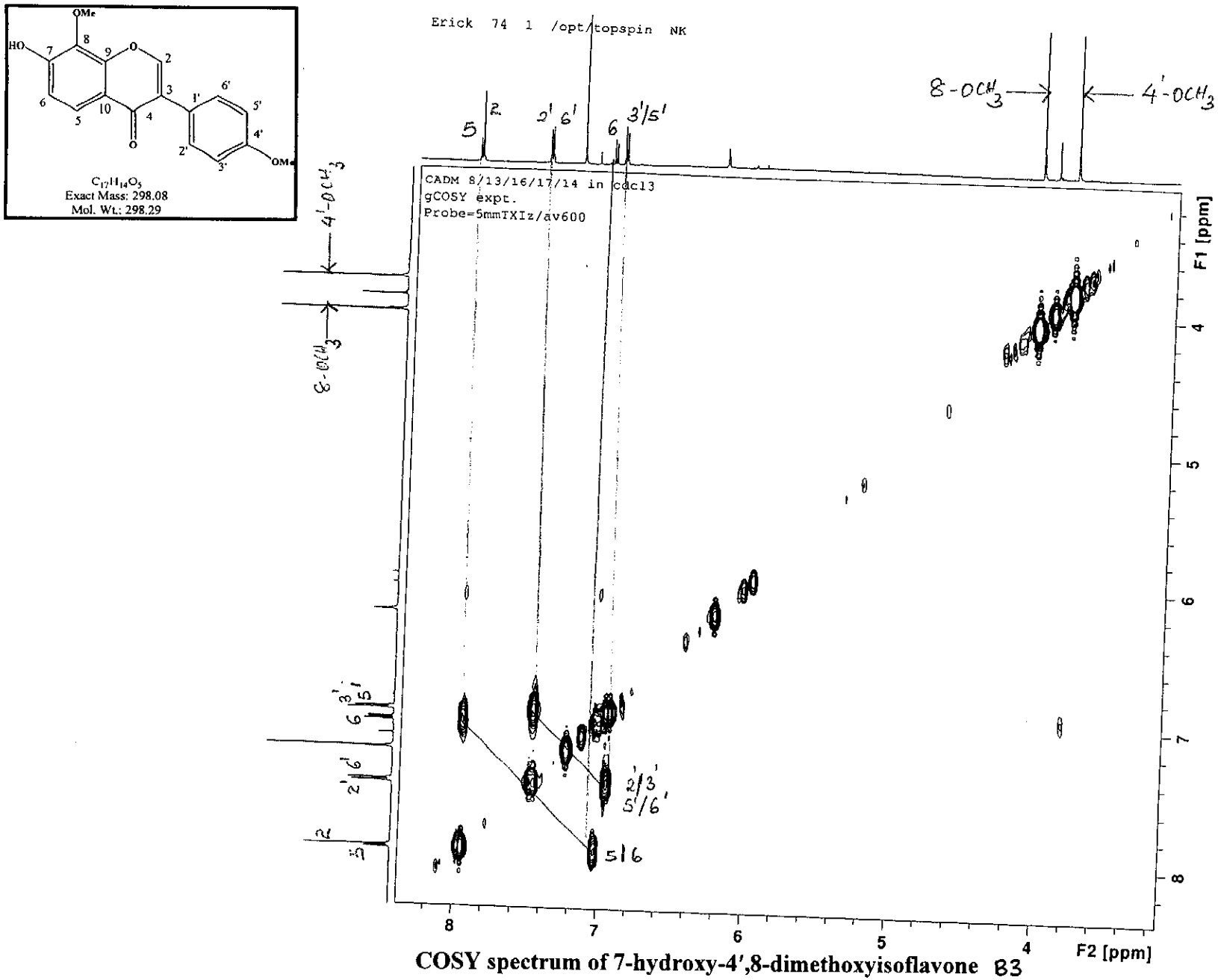


^{13}C NMR spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone

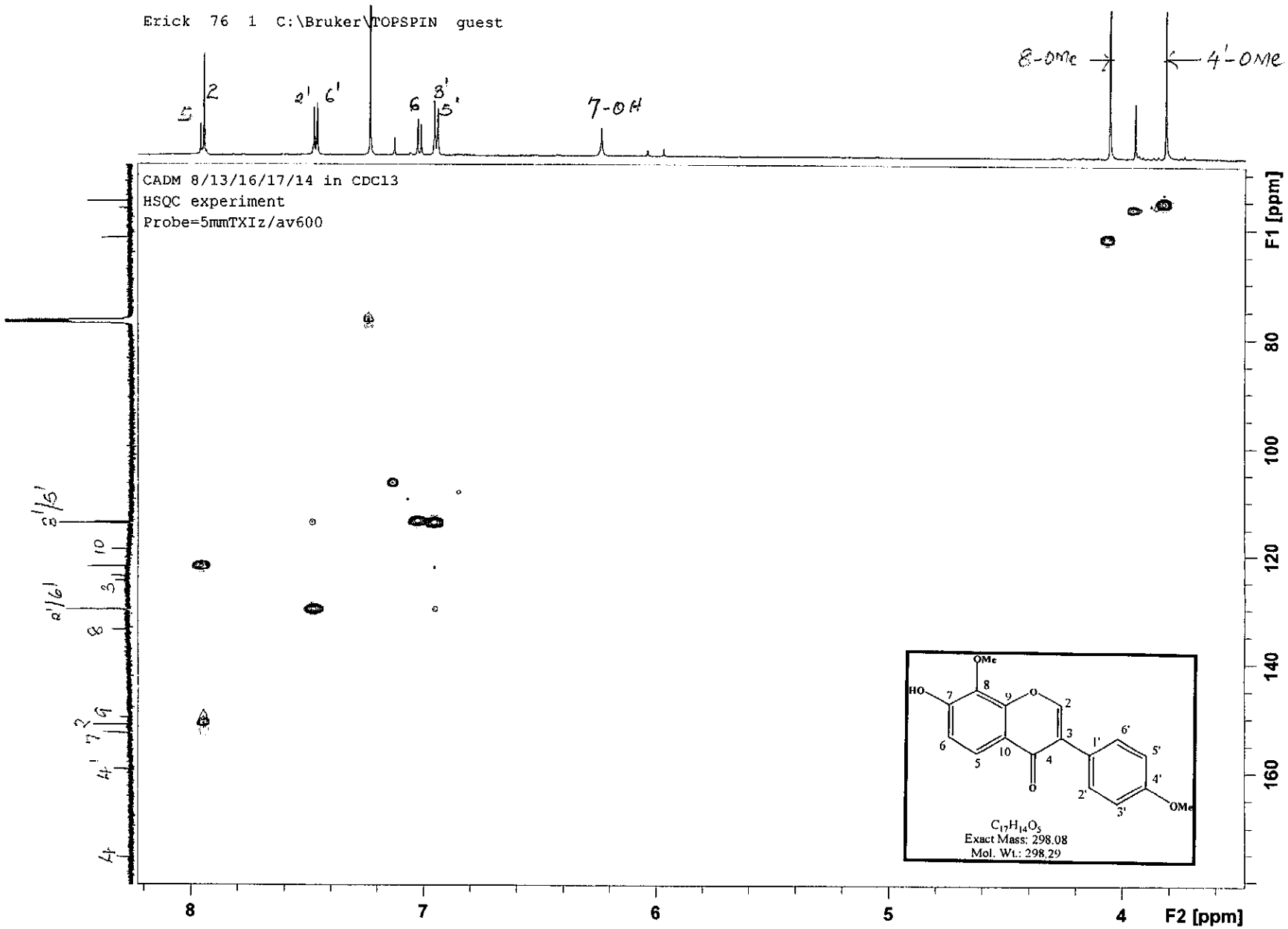
Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity	Annotation
1	175.7734	26523.3738	0.10	
2	159.6932	24096.9477	0.12	
3	152.9474	23079.0385	0.20	
4	151.4094	22846.9615	0.28	
5	149.9743	22630.4117	0.08	
6	133.8975	20204.4987	0.14	
7	130.1911	19645.2205	0.51	
8	124.8297	18836.2107	0.12	
9	123.9095	18697.3568	0.15	
10	122.2238	18442.9927	0.32	
11	119.0448	17963.2966	0.14	
12	114.0181	17204.7914	0.55	
13	113.7705	17167.4297	0.27	
14	77.2297	11653.5960	14.87	
15	77.0182	11621.6817	15.00	
16	76.8065	11589.7372	14.82	
17	61.9685	9350.7532	0.22	
18	56.4906	8524.1641	0.08	
19	55.3588	8353.3808	0.32	



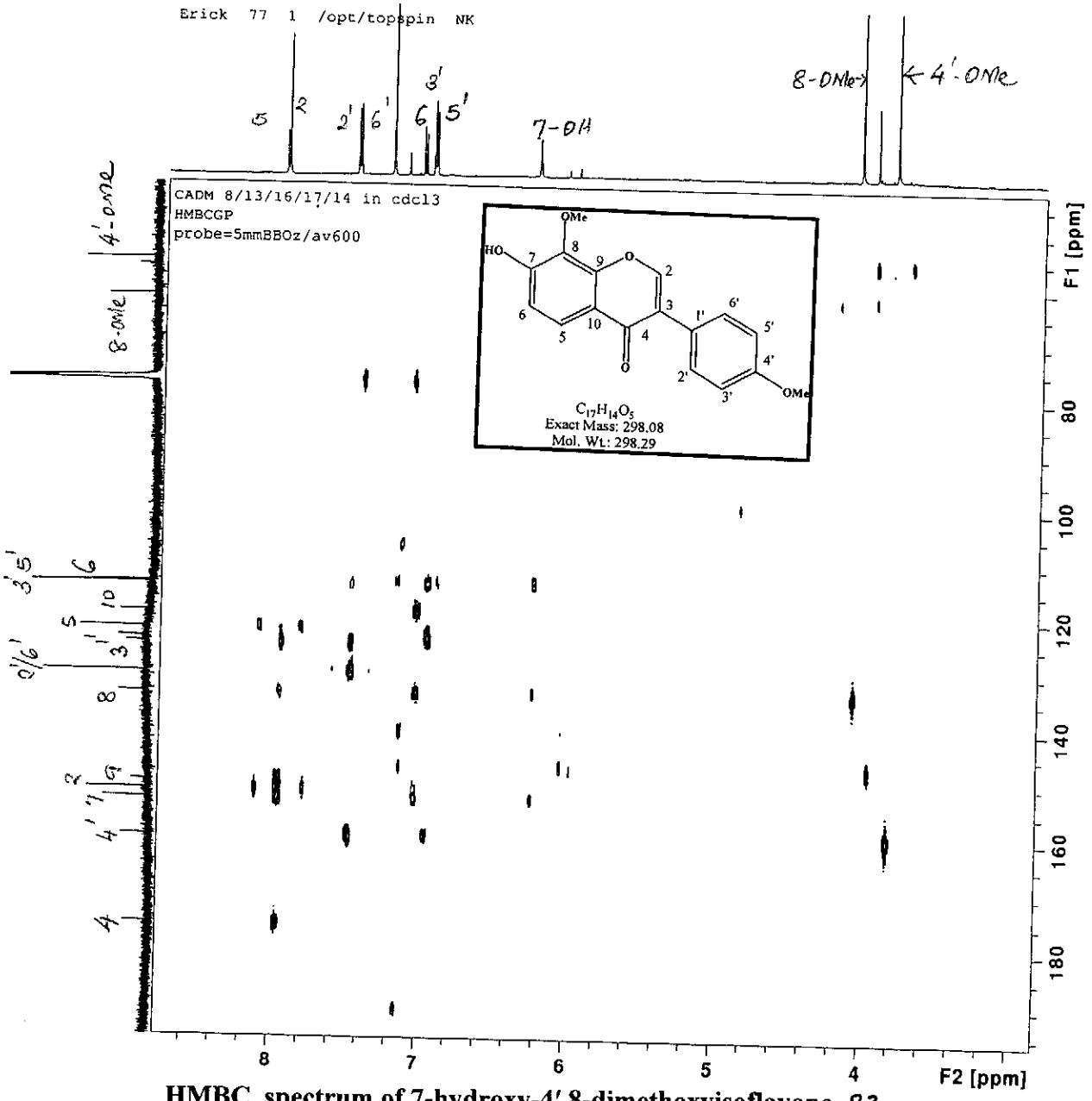
DEPT spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone B3



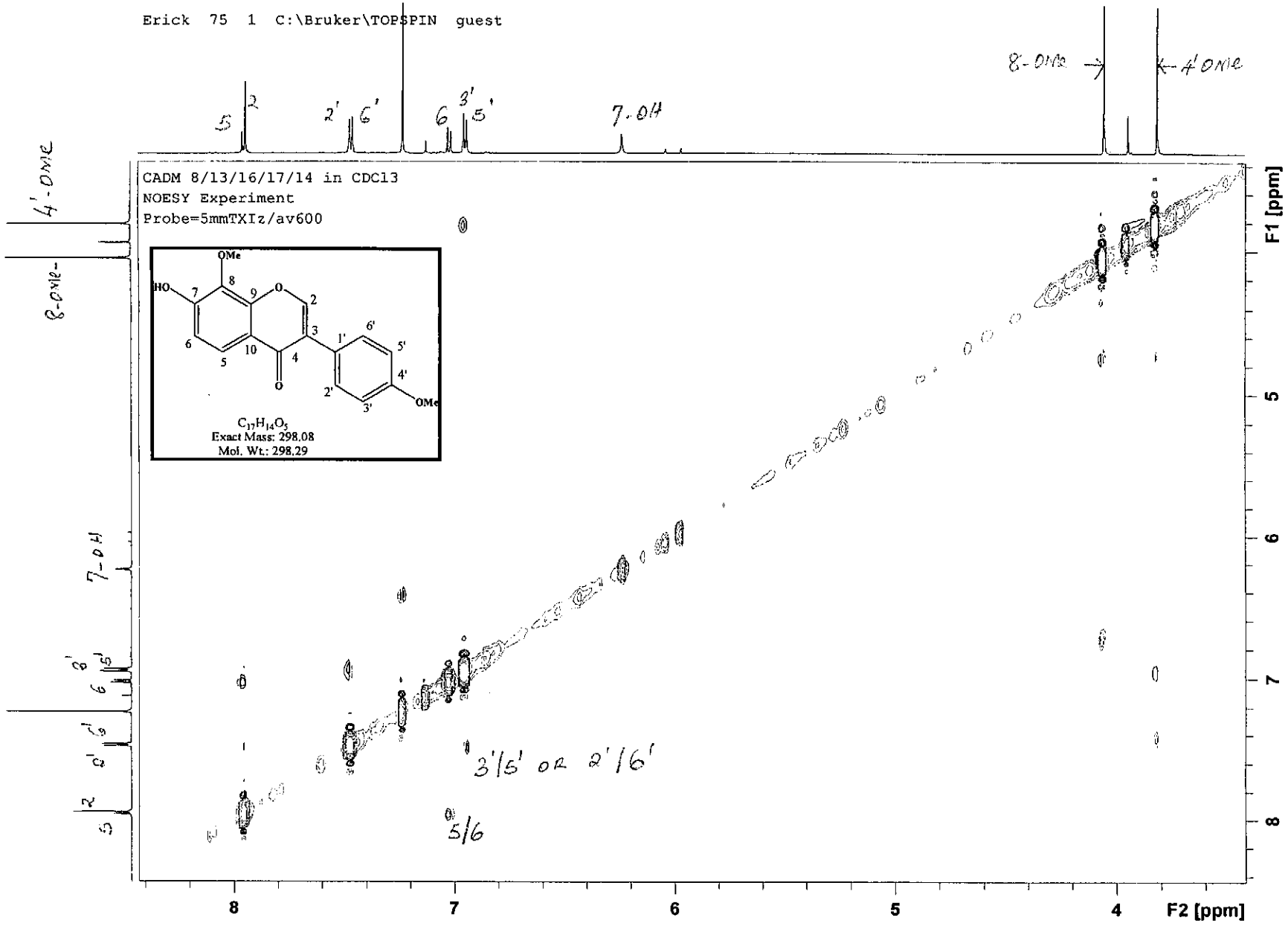
COSY spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone



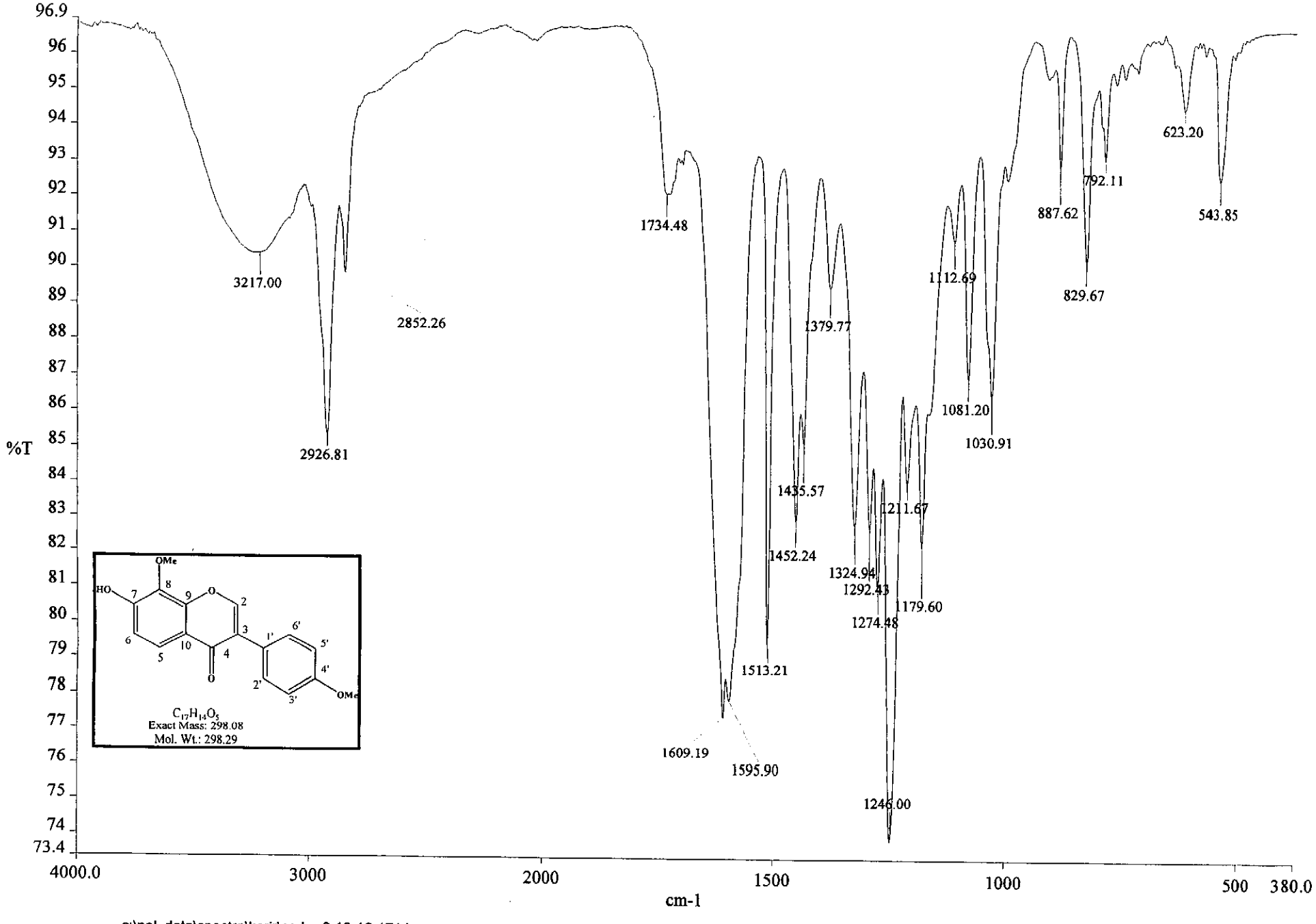
HSQC spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone 83



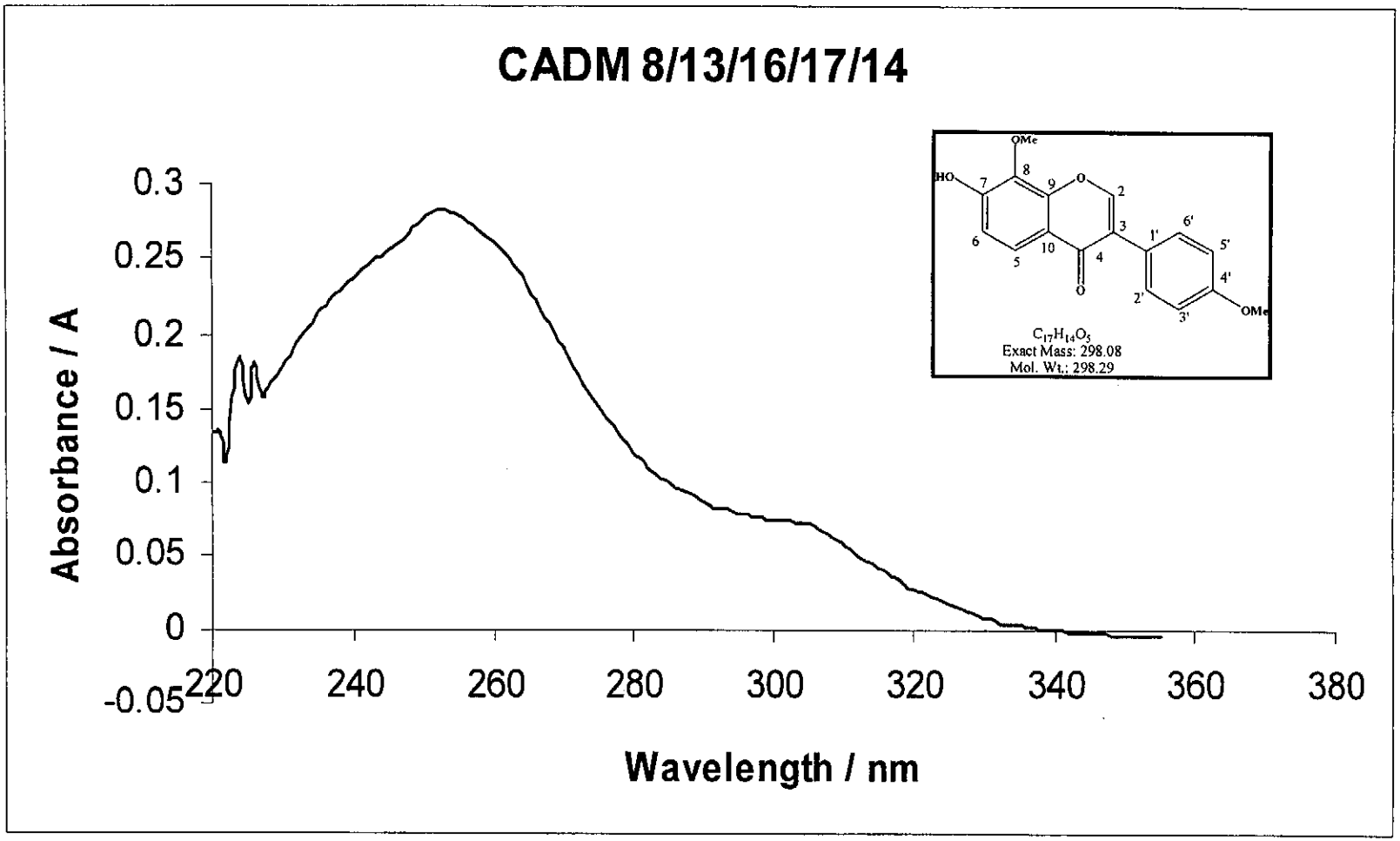
HMBC spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone B3



NOESY spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone B3

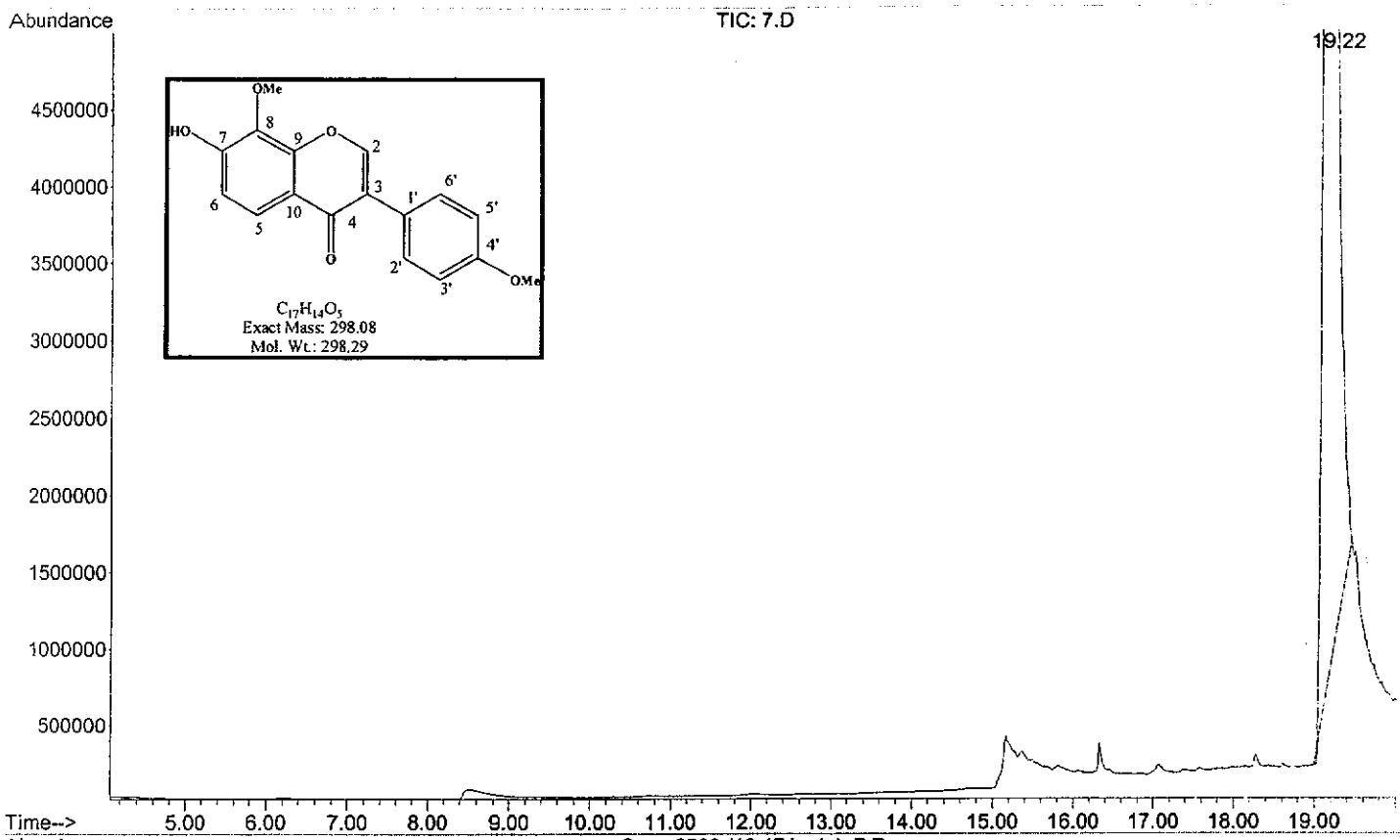


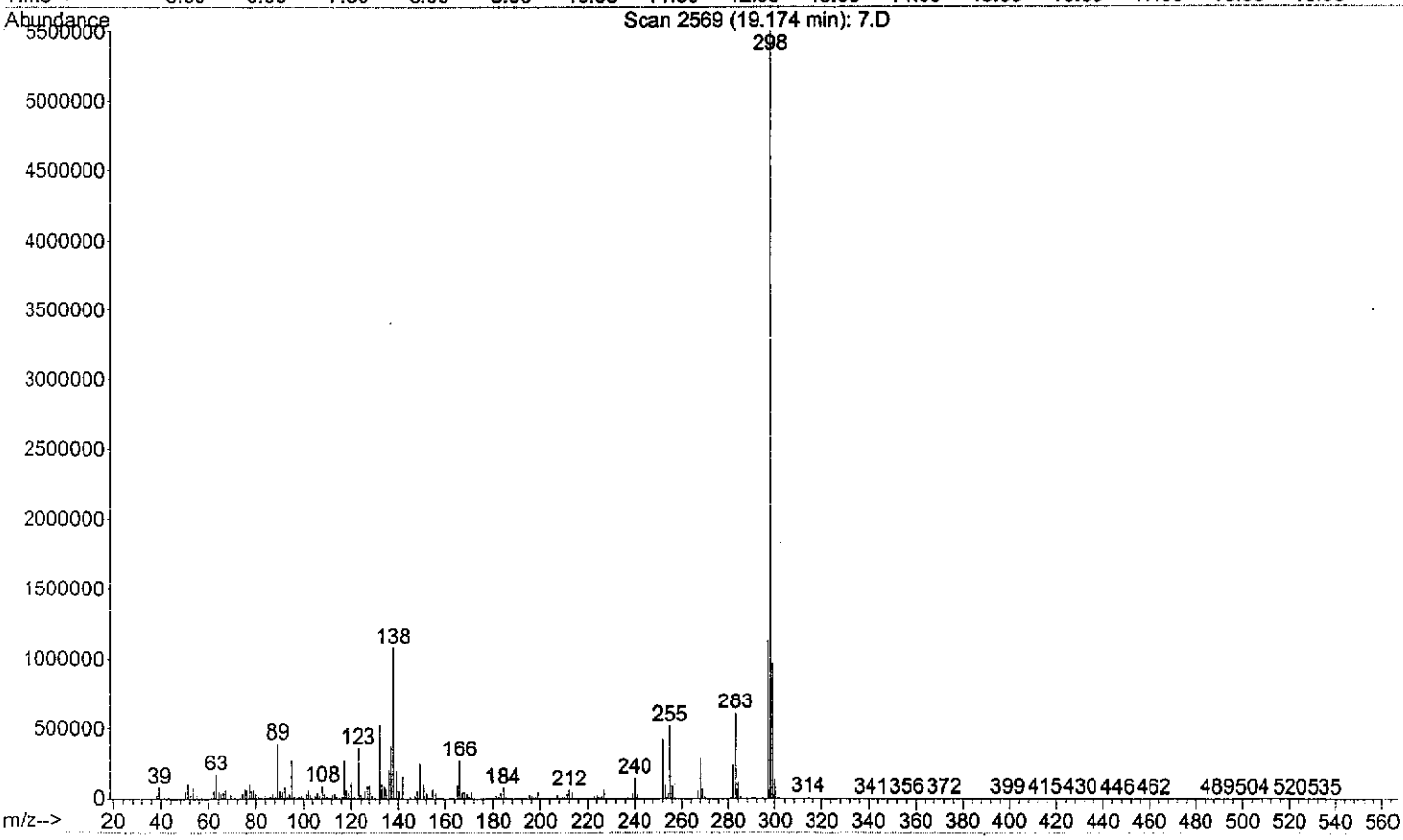
IR spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone B3



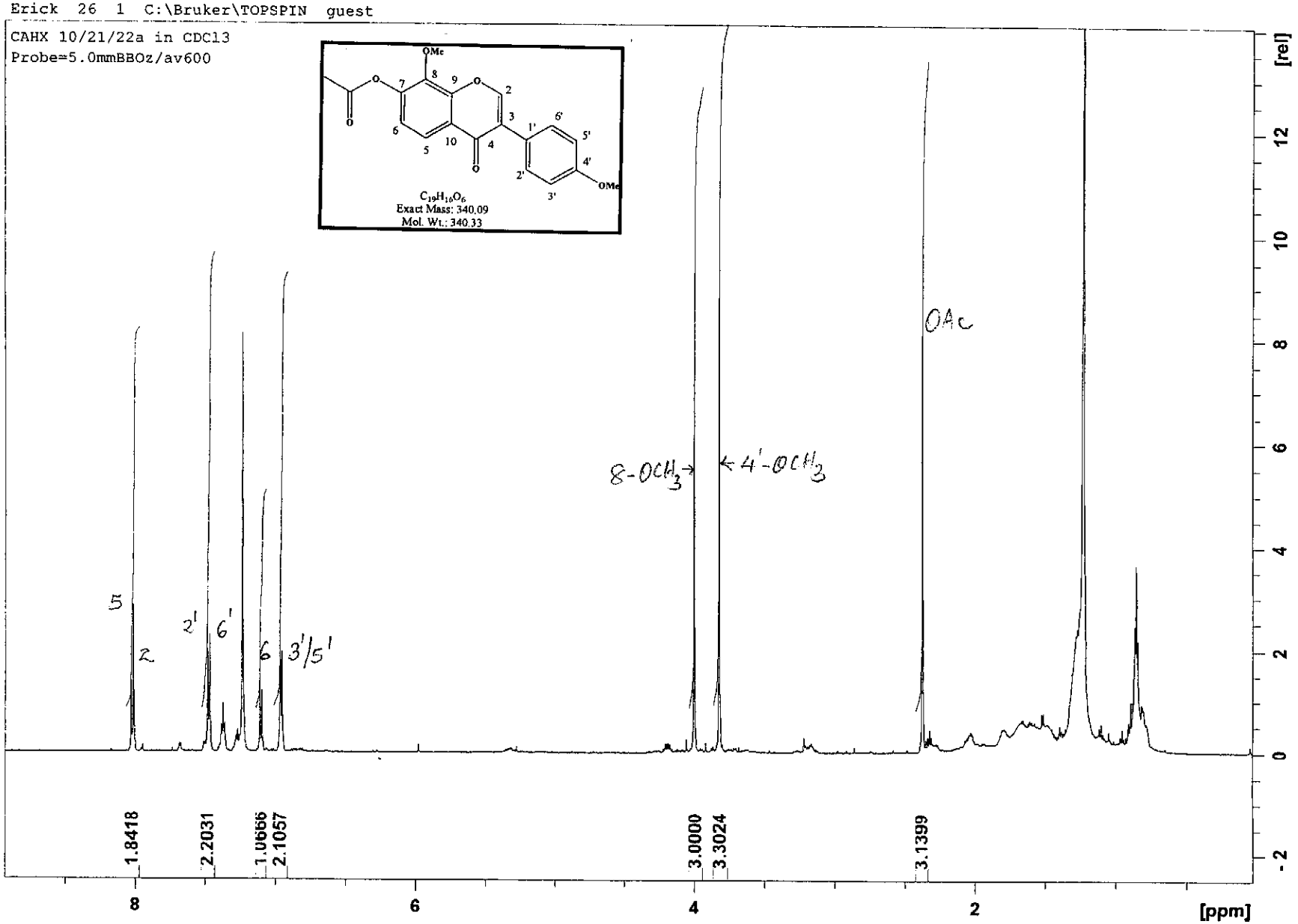
UV spectrum of 7-hydroxy-4',8-dimethoxyisoflavone 83

File : D:\ERICK2009\7.D
Operator : ERICK
Acquired : 4 Sep 2009 22:00 using AcqMethod ERICK2
Instrument : Instrumen
Sample Name: 040909 RERUN 3ULCHANGED OVEN TEMP
Misc Info : CADM 8\13\16\17\14
Vial Number: 1



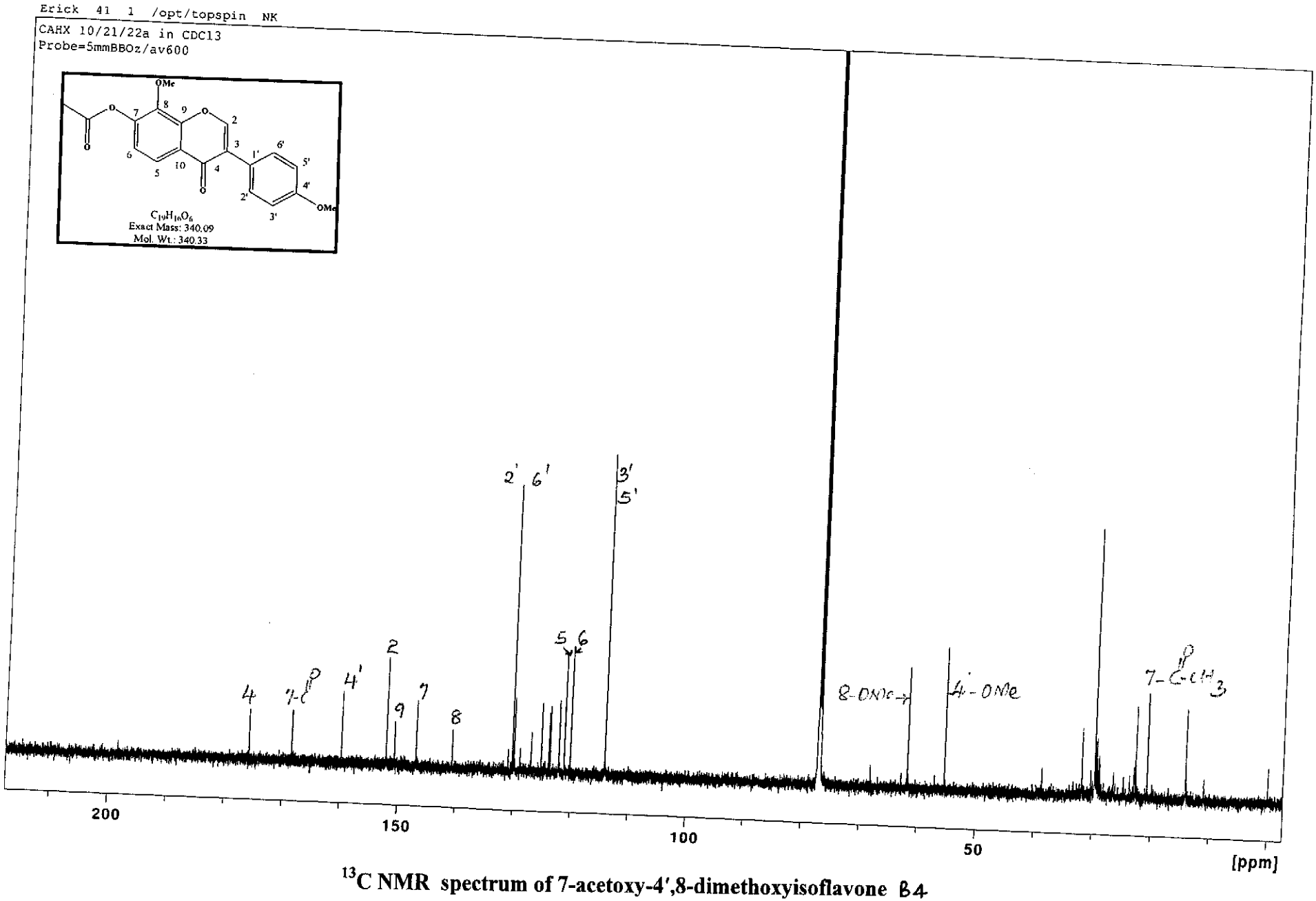


Mass spectrum of 7-hydroxy-4′,8-dimethoxyisoflavone B3



^{1}H NMR spectrum of 7-acetoxy-4′,8-dimethoxyisoflavone B4

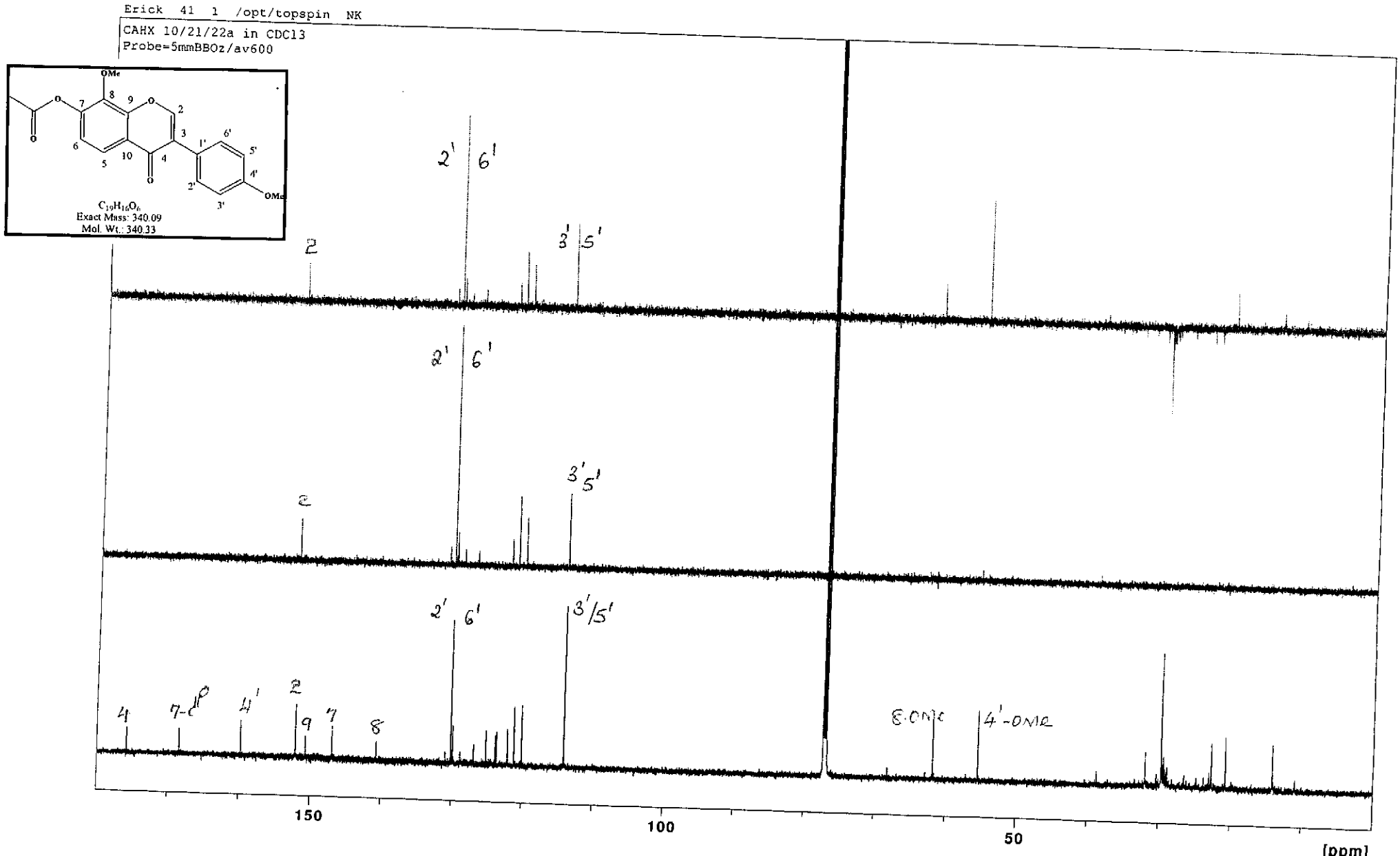
Peak	ν(F1) [ppm]	Intensity [abs]	Annotation
1	8.0284	432289.73	
2	8.0119	1309432.36	
3	7.4829	851361.72	
4	7.4688	1047984.05	
5	7.2354	3752287.67	
6	7.1113	448929.11	
7	7.0968	569604.61	
8	6.9681	1044028.02	
9	6.9537	888774.95	
10	4.0010	3967350.17	
11	3.8241	4077917.55	
12	2.3736	3747059.77	
13	1.2799	1050989.25	
14	1.2700	1112957.25	
15	1.2569	1272495.77	
16	1.2263	6869741.42	
17	0.8644	1139599.84	
18	0.8531	1686029.84	
19	0.8411	1008189.05	



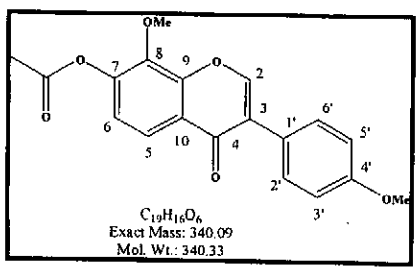
^{13}C NMR spectrum of 7-acetoxy-4′,8-dimethoxyisoflavone B4

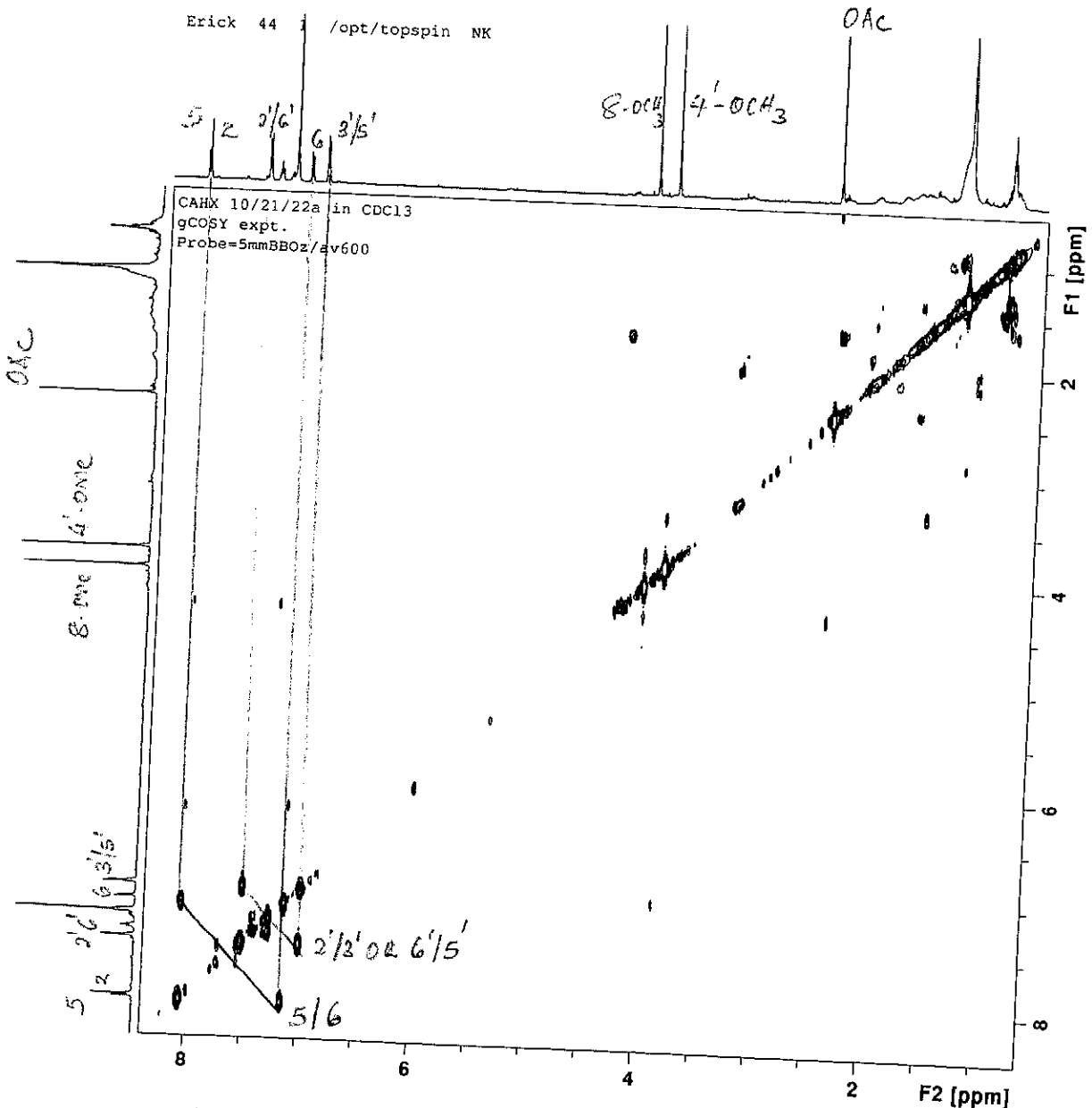
Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity	Annotation
1	175.7810	26524.5206	0.13	
2	168.4092	25412.1509	0.13	
3	159.8086	24114.3610	0.18	
4	152.0569	22944.6662	0.27	
5	150.6722	22735.7215	0.11	
6	146.9163	22168.9740	0.16	
7	140.6610	21225.0789	0.10	
8	130.8883	19750.4247	0.05	
9	130.1498	19638.9886	0.71	
10	129.9533	19609.3376	0.05	
11	129.8443	19592.8901	0.18	
12	128.8092	19436.6984	0.06	
13	126.8848	19146.3155	0.09	
14	125.1332	18882.0074	0.16	
15	123.8056	18681.6788	0.15	
16	123.6161	18653.0842	0.16	
17	122.0715	18420.0113	0.17	
18	121.1452	18280.2371	0.29	
19	120.0886	18120.8011	0.30	
20	114.0777	17213.7848	0.79	
21	77.2296	11653.5810	14.92	
22	76.8064	11589.7221	14.84	
23	68.1697	10286.4849	0.05	
24	61.7540	9318.3862	0.29	
25	55.3658	8354.4371	0.35	
26	38.7337	5844.7319	0.06	
27	31.9289	4817.9198	0.16	
28	30.3665	4582.1611	0.06	
29	29.7042	4482.2231	0.65	
30	29.6656	4476.3986	0.20	
31	29.5966	4465.9868	0.13	
32	29.5313	4456.1333	0.09	
33	29.4864	4449.3581	0.11	
34	29.4495	4443.7901	0.06	
35	29.3661	4431.2054	0.14	
36	29.3033	4421.7292	0.09	
37	29.2550	4414.4410	0.08	
38	29.1867	4404.1348	0.05	
39	29.0782	4387.7627	0.06	
40	28.9311	4365.5660	0.10	
41	28.8596	4354.7770	0.09	
42	26.4781	3995.4199	0.06	
43	24.7503	3734.7031	0.05	
44	23.7492	3583.6418	0.05	
45	22.9885	3468.8558	0.07	

Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity	Annotation
46	22.6958	3424.6888	0.22	
47	20.7320	3128.3606	0.27	
48	14.1229	2131.0787	0.22	
49	14.0550	2120.8329	0.07	
50	10.9644	1654.4760	0.05	
51	-0.0038	-0.5734	0.08	

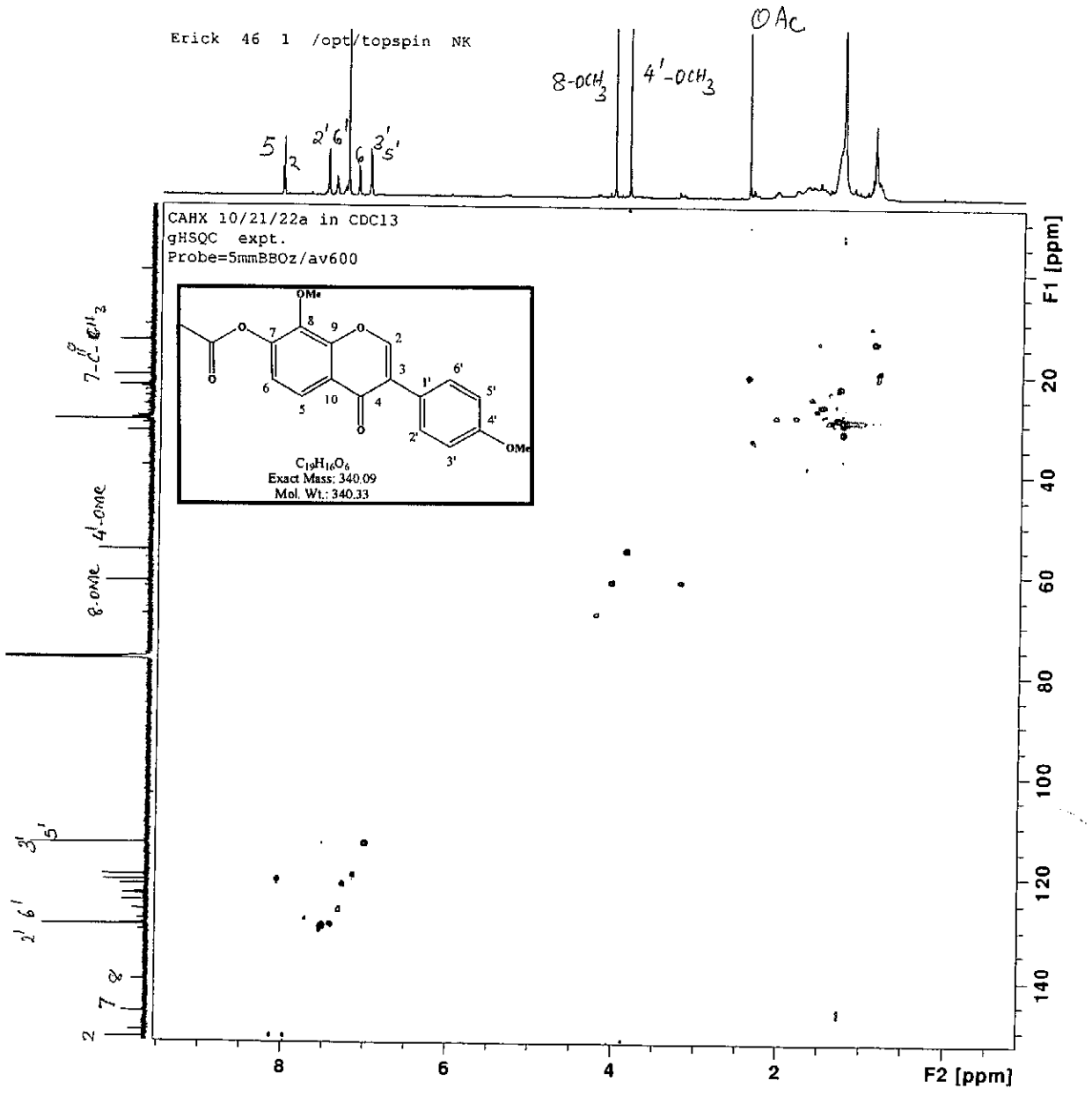


DEPT spectrum of 7-acetoxy-4′,8-dimethoxyisoflavone β4

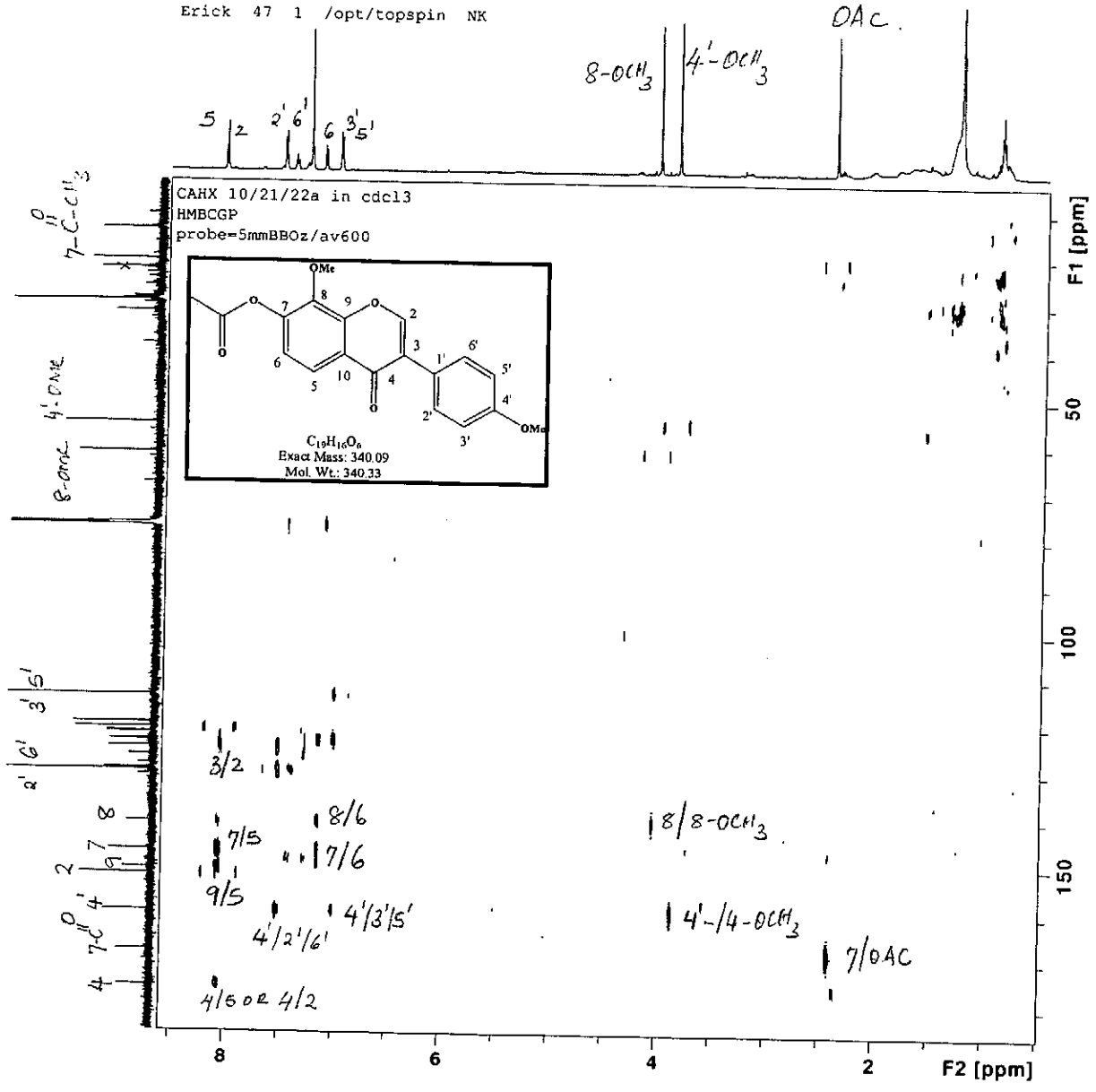




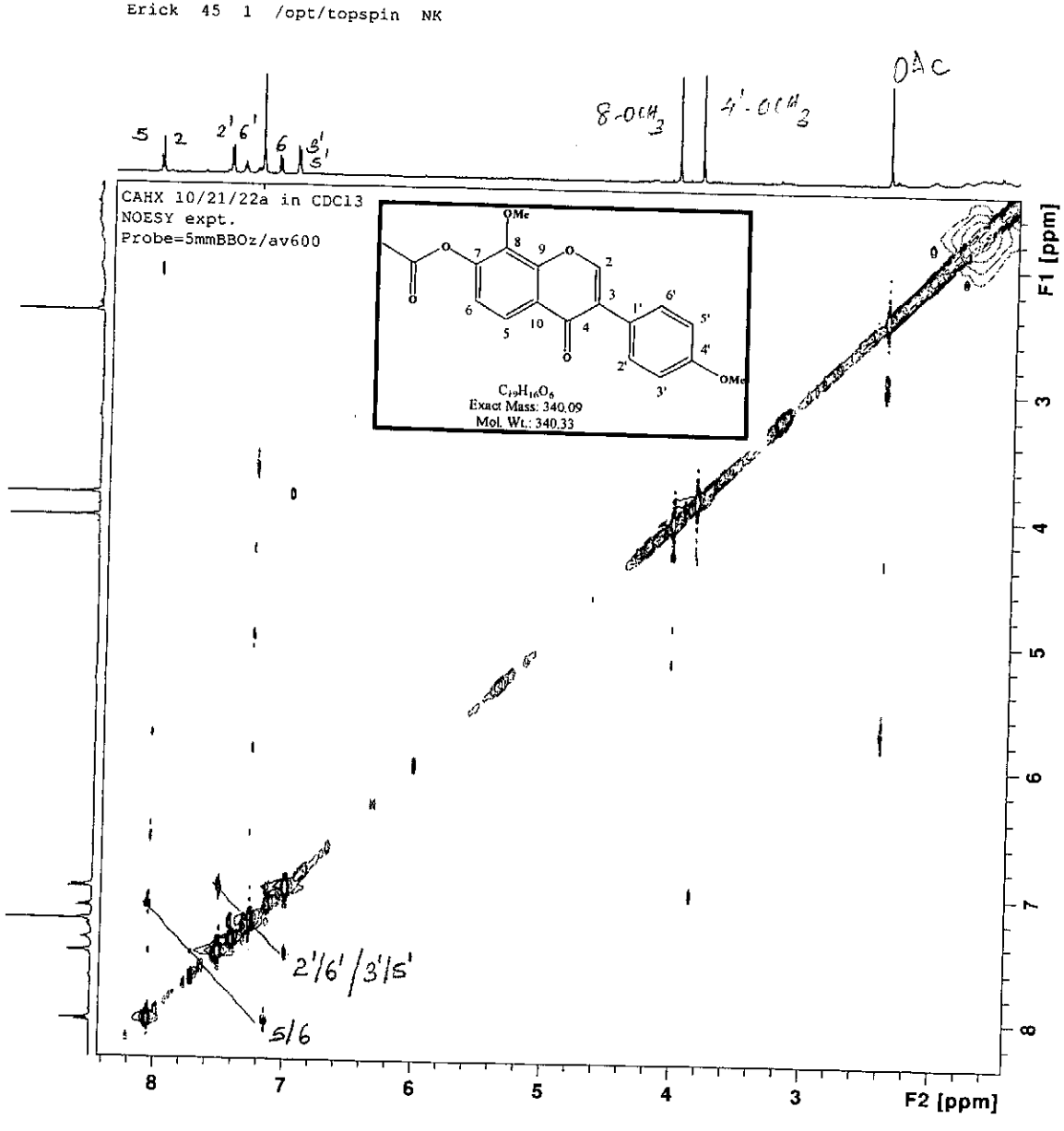
COSY spectrum of 7-acetoxy-4′,8-dimethoxyisoflavone B4



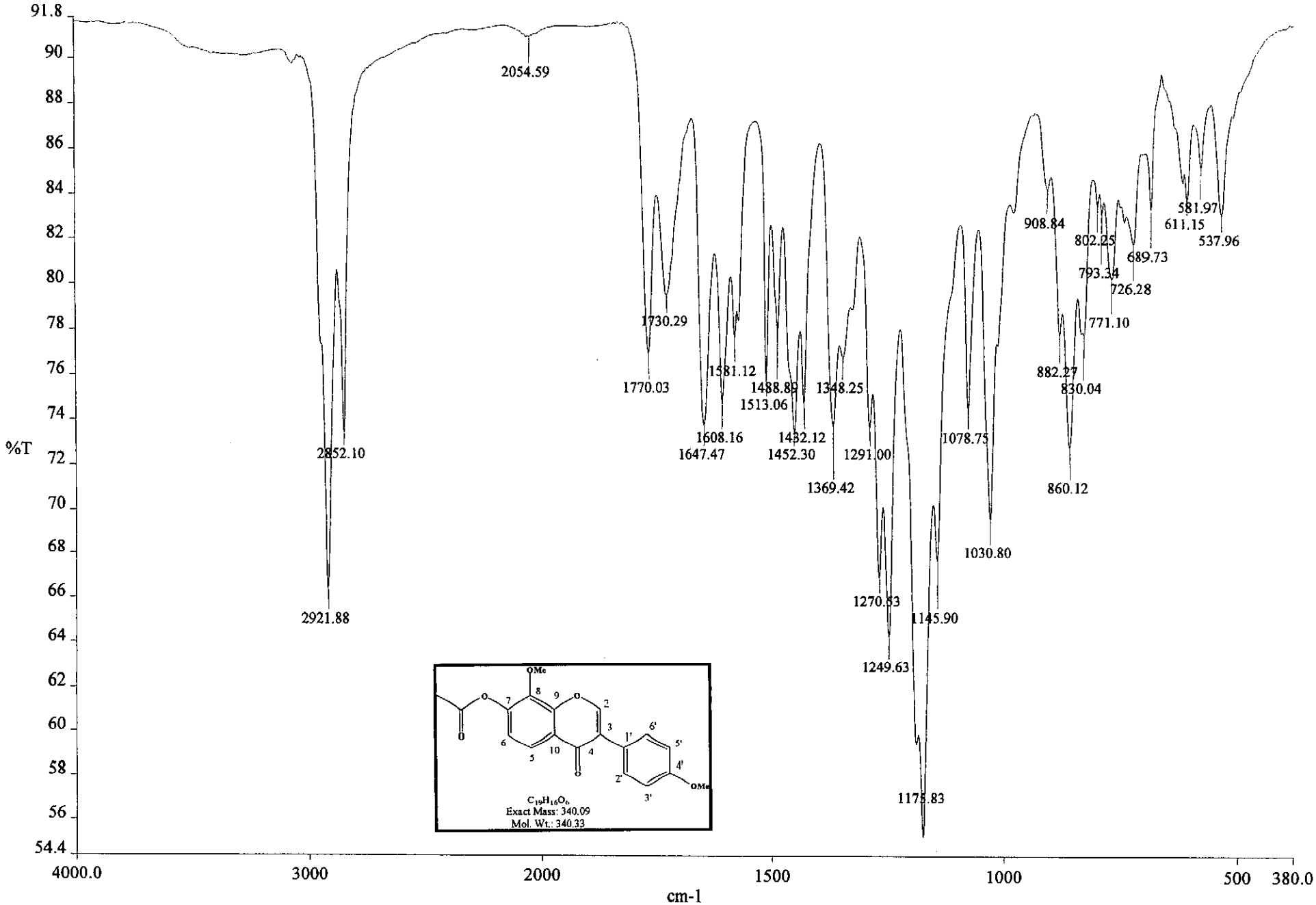
HSQC spectrum of 7-acetoxy-4′,8-dimethoxyisoflavone B4



HMBC spectrum of 7-acetoxy-4′,8-dimethoxyisoflavone B4

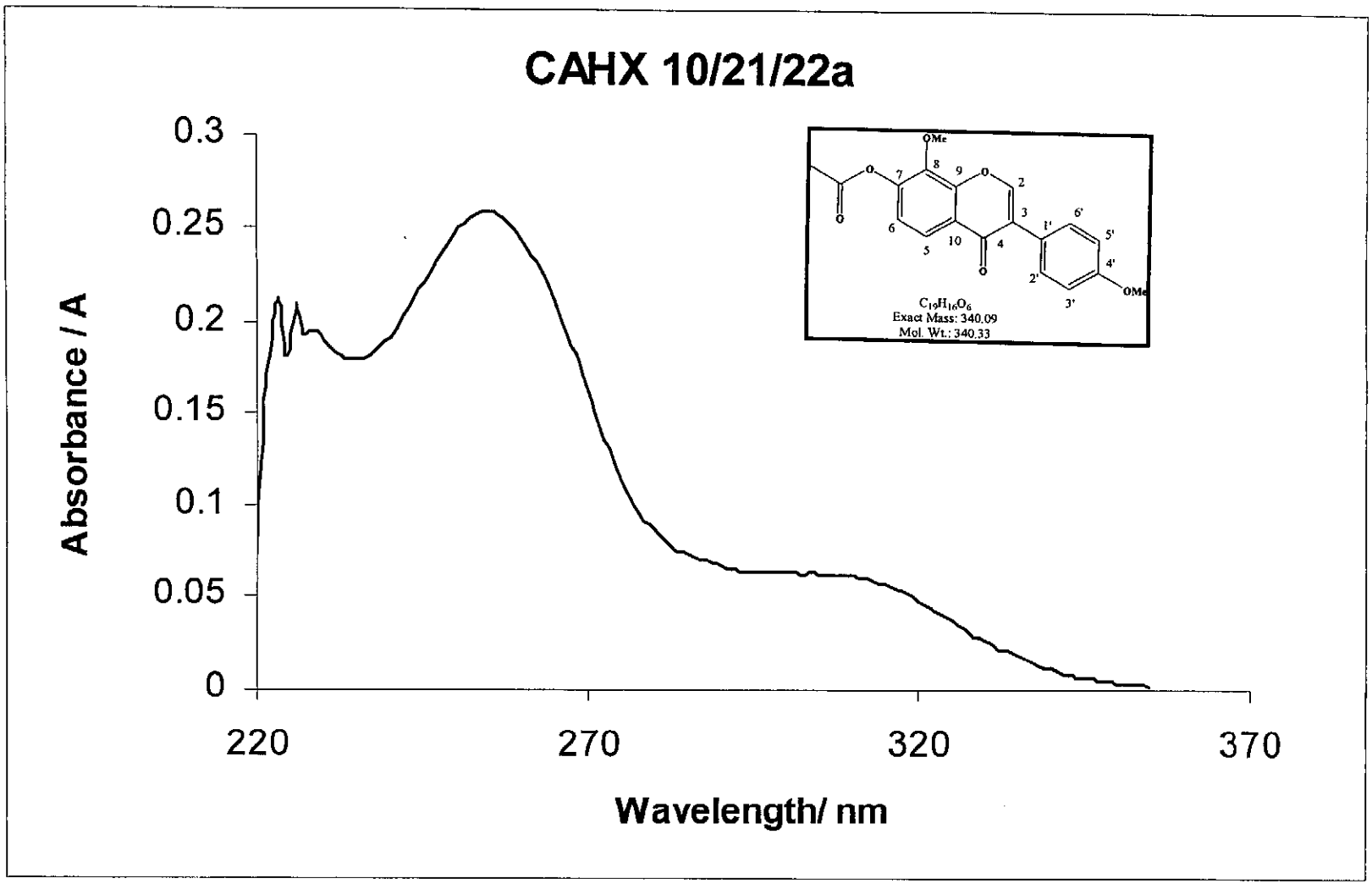


NOESYspectrum of 7-acetoxy-4′,8-dimethoxyisoflavone 84



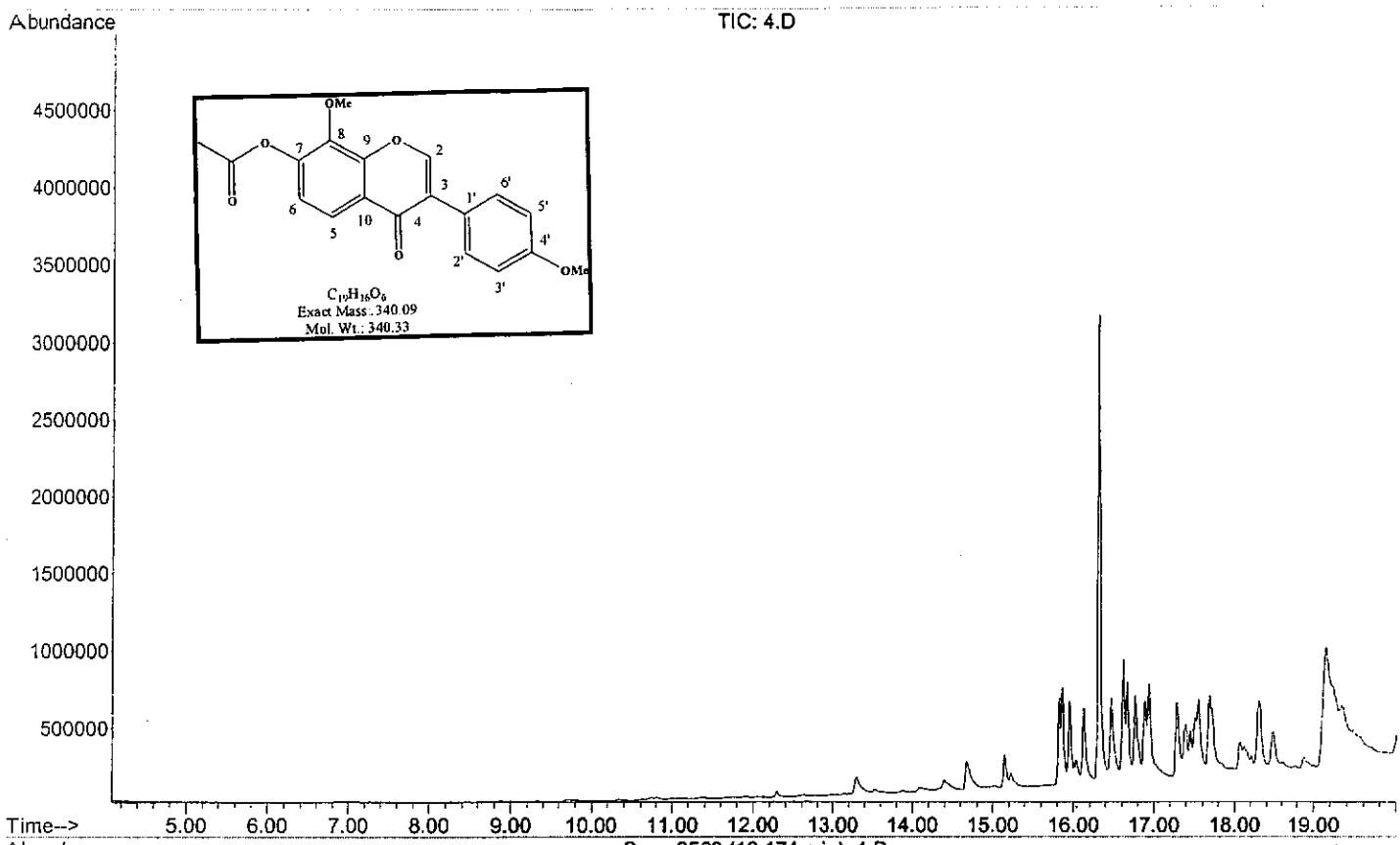
c:\pel_data\spectra\cahx 10-21-22a.002

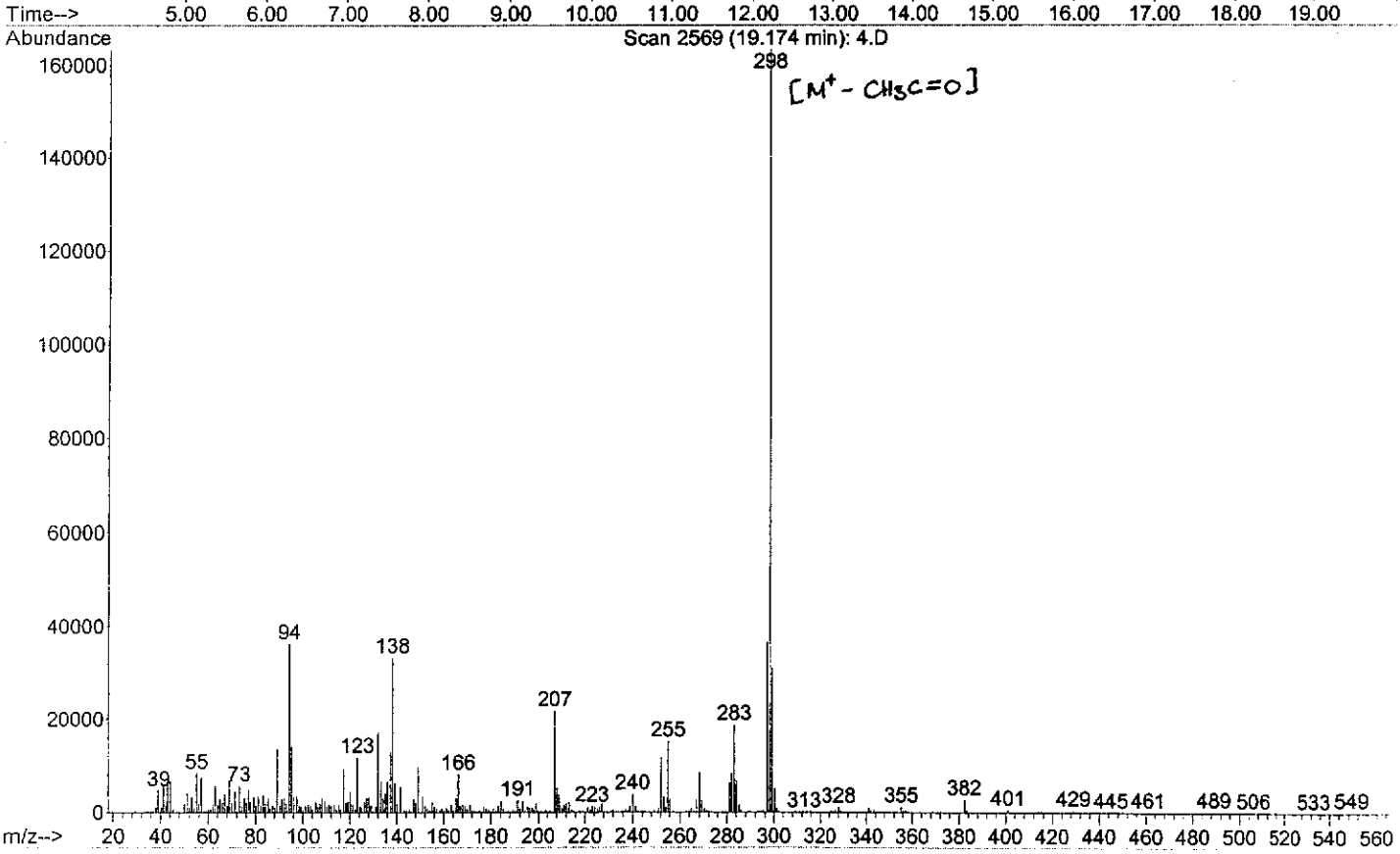
IR spectrum of 7-acetoxy-4',8-dimethoxyisoflavone B4



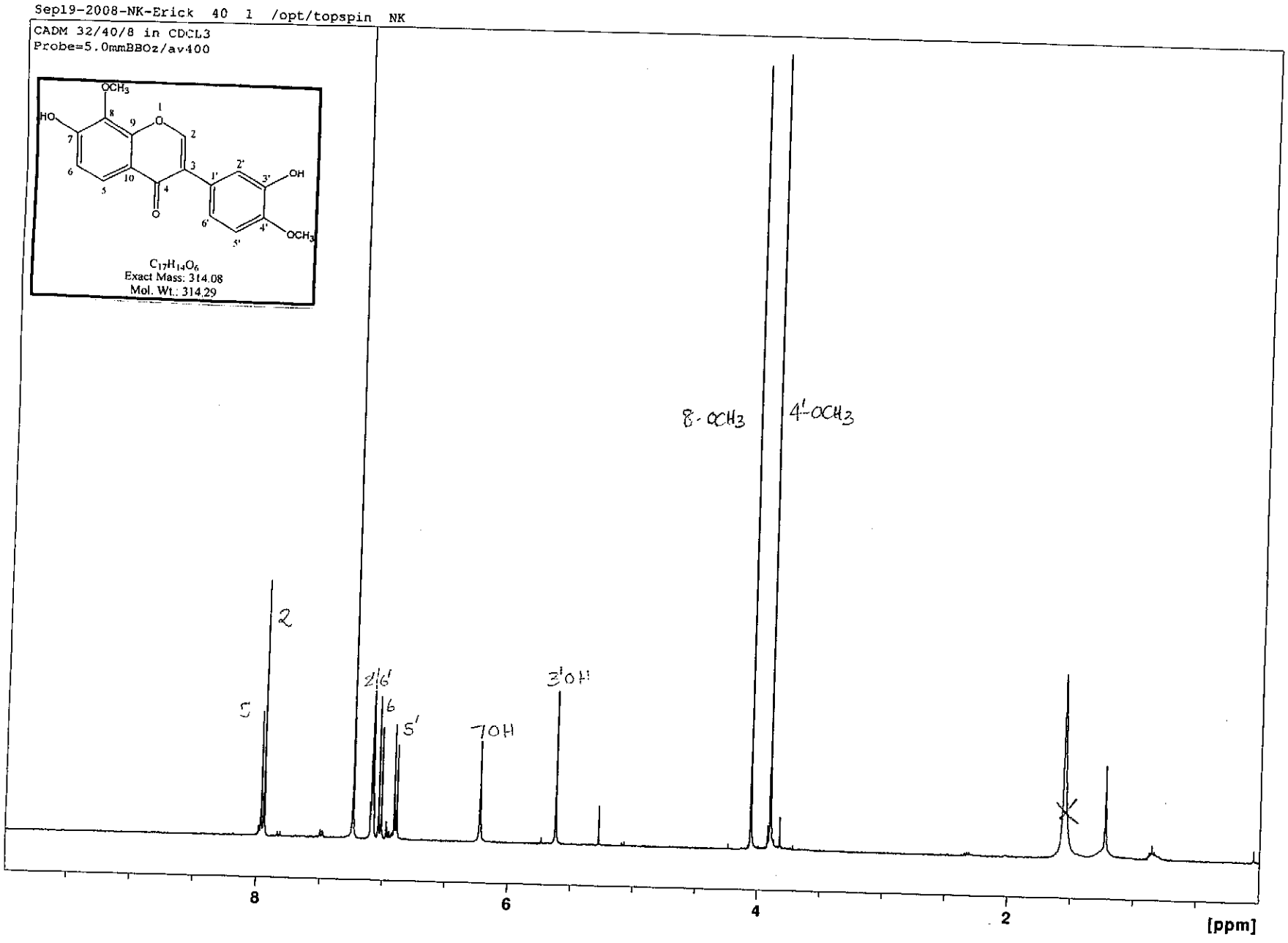
UV spectrum of 7-acetoxy-4',8-dimethoxyisoflavone B4

File : D:\ERICK2009\4.D
Operator : ERICK
Acquired : 4 Sep 2009 20:45 using AcqMethod ERICK2
Instrument : Instrumen
Sample Name: 040909 RERUN 3ULCHANGED OVEN TEMP
Misc Info : CAHX 10/21/229
Vial Number: 1



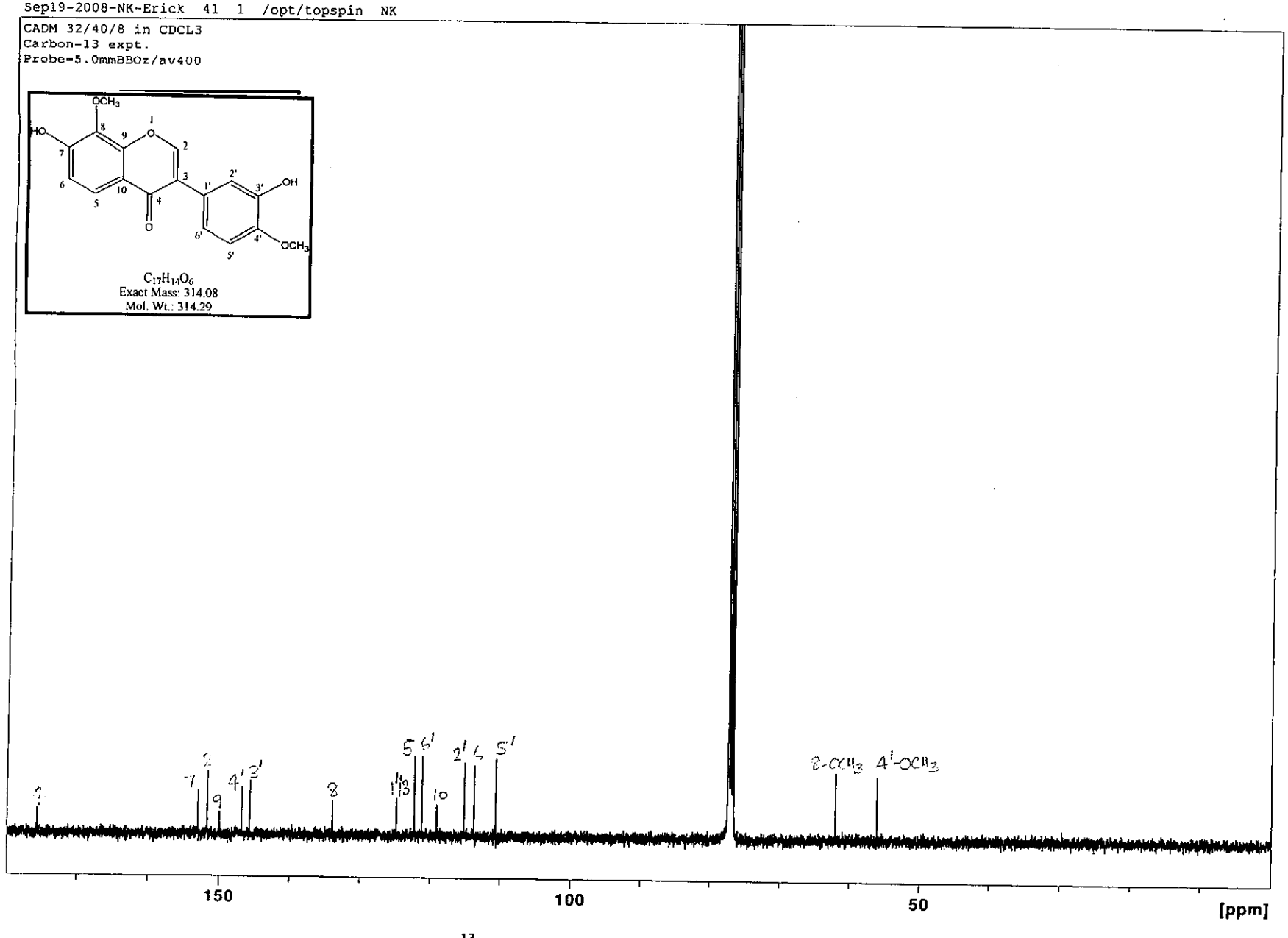


Mass spectrum of 7-acetoxy-4′,8-dimethoxyisoflavone B4



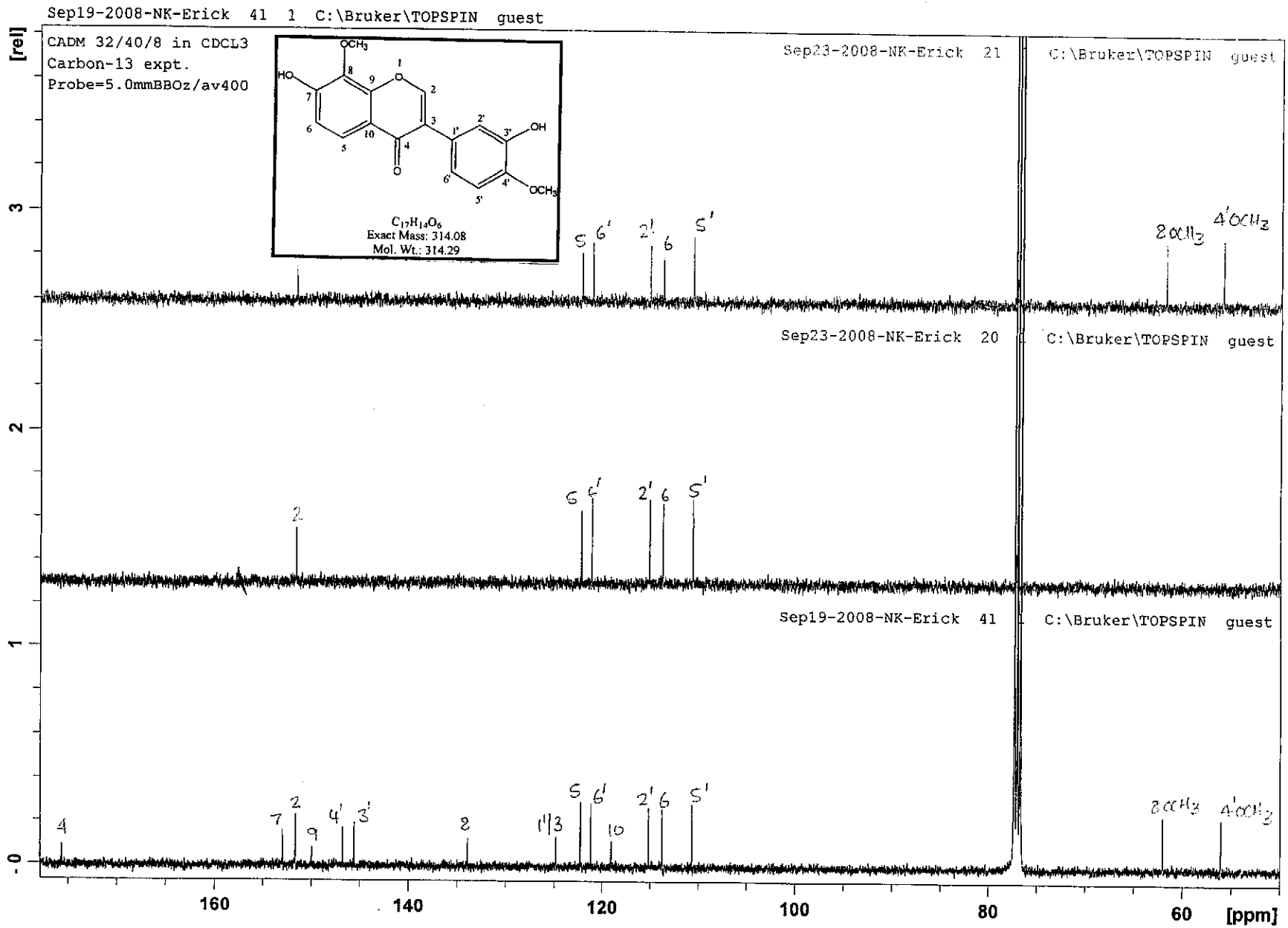
^{1}H NMR spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone 85

Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity [rel]	Annotation
2	7.9395	3177.5468	2.31	
4	7.2337	2895.0715	15.00	
6	7.0911	2838.0002	1.16	
8	7.0796	2833.3976	2.00	
10	7.0694	2829.3154	0.61	
12	7.0085	2804.9420	1.58	
14	6.9075	2764.5198	1.55	
16	6.8852	2755.5949	1.26	
18	5.6201	2249.2765	2.12	
20	4.0560	1623.2924	10.79	
22	3.9002	1560.9381	10.72	
24	1.5532	621.6217	2.47	
26	0.8513	340.7073	0.20	

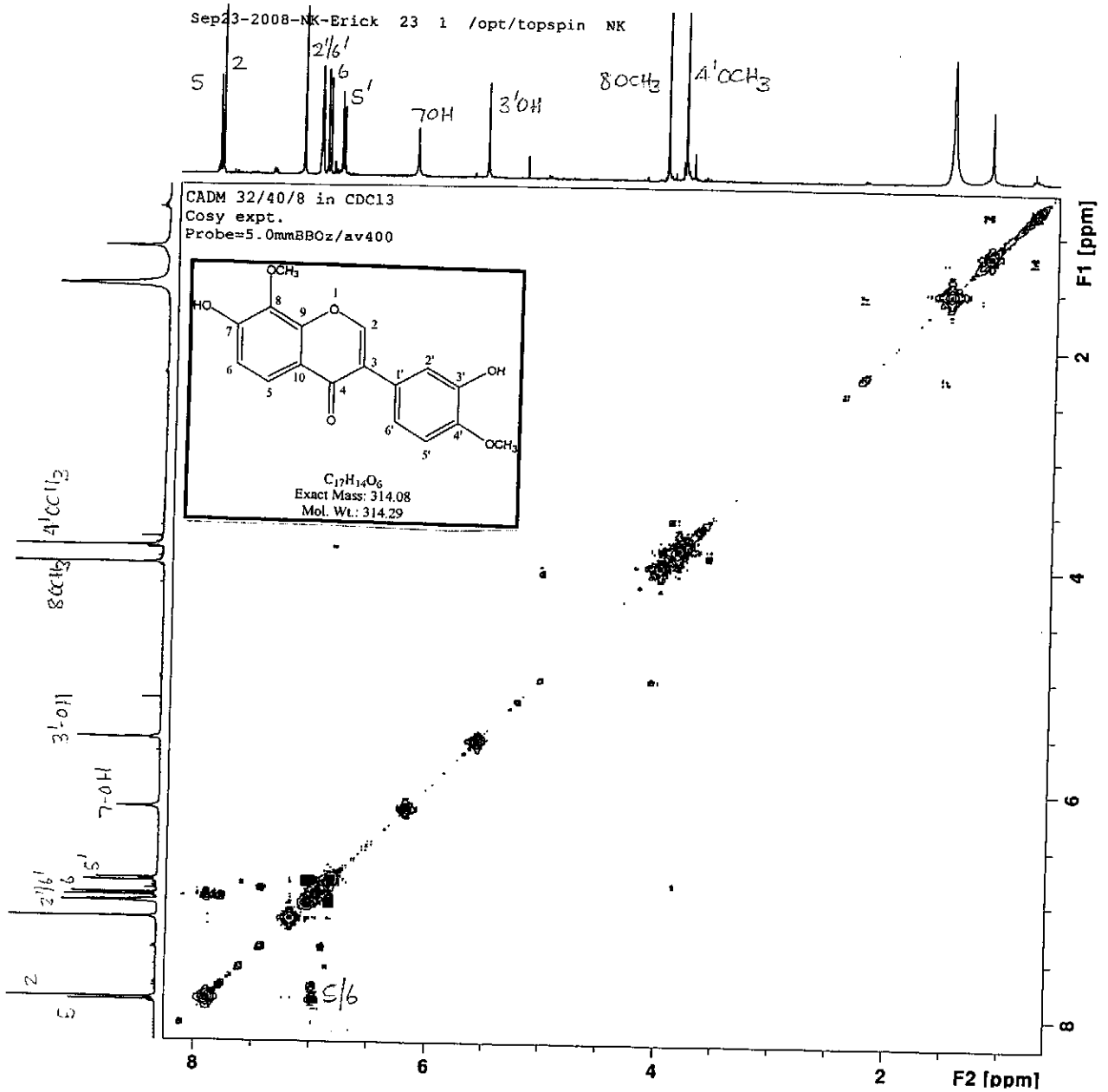


^{13}C NMR spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone 85

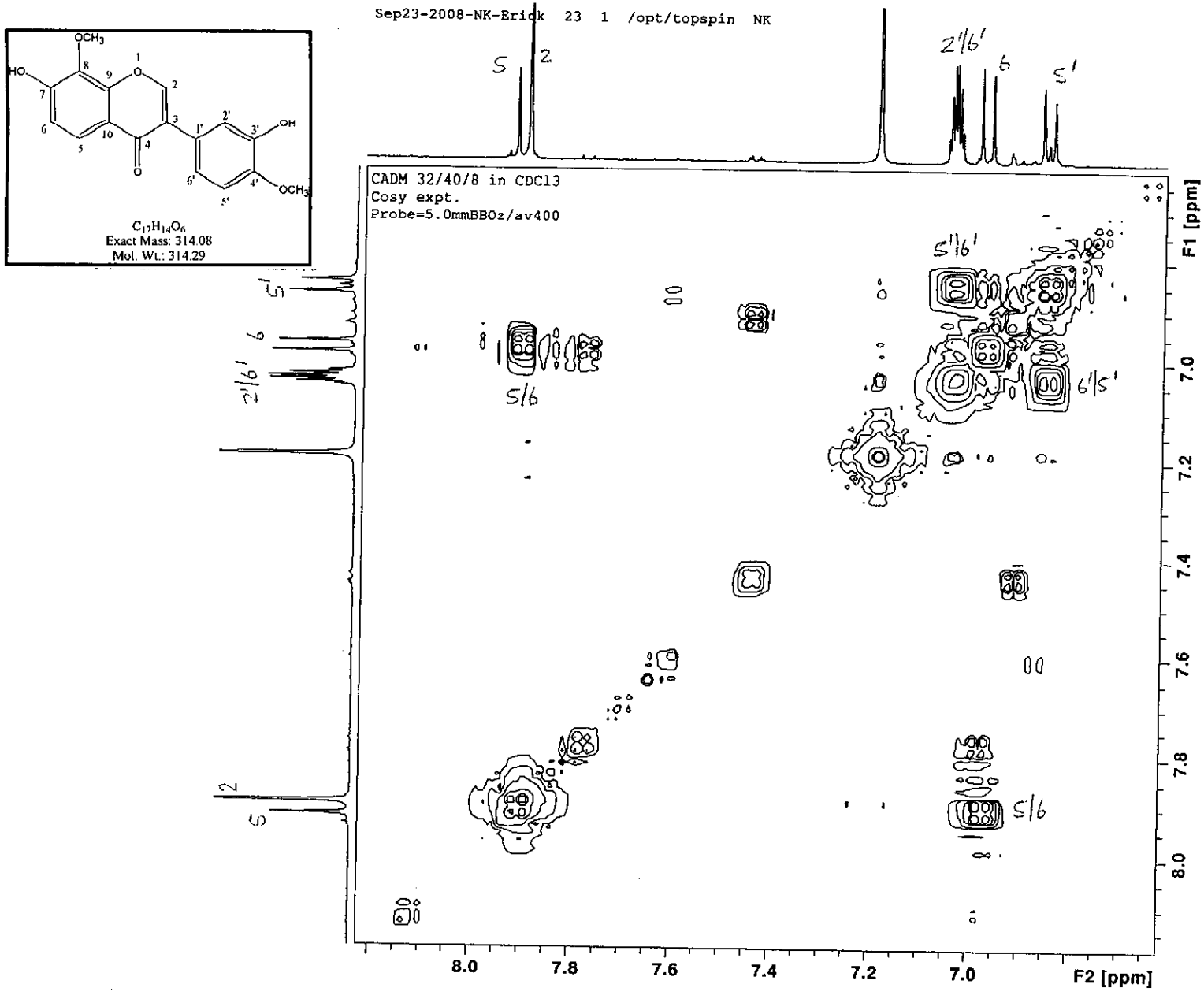
Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity [rel]	Annotation
2	152.9360	15390.7754	0.17	
4	149.9148	15086.7357	0.09	
6	145.5800	14650.5014	0.20	
8	124.8347	12562.7898	0.14	
10	122.2447	12302.1442	0.30	
12	119.0519	11980.8355	0.12	
14	113.7587	11448.1522	0.27	
16	77.2273	7771.8001	0.58	
18	56.0430	5639.9097	0.25	



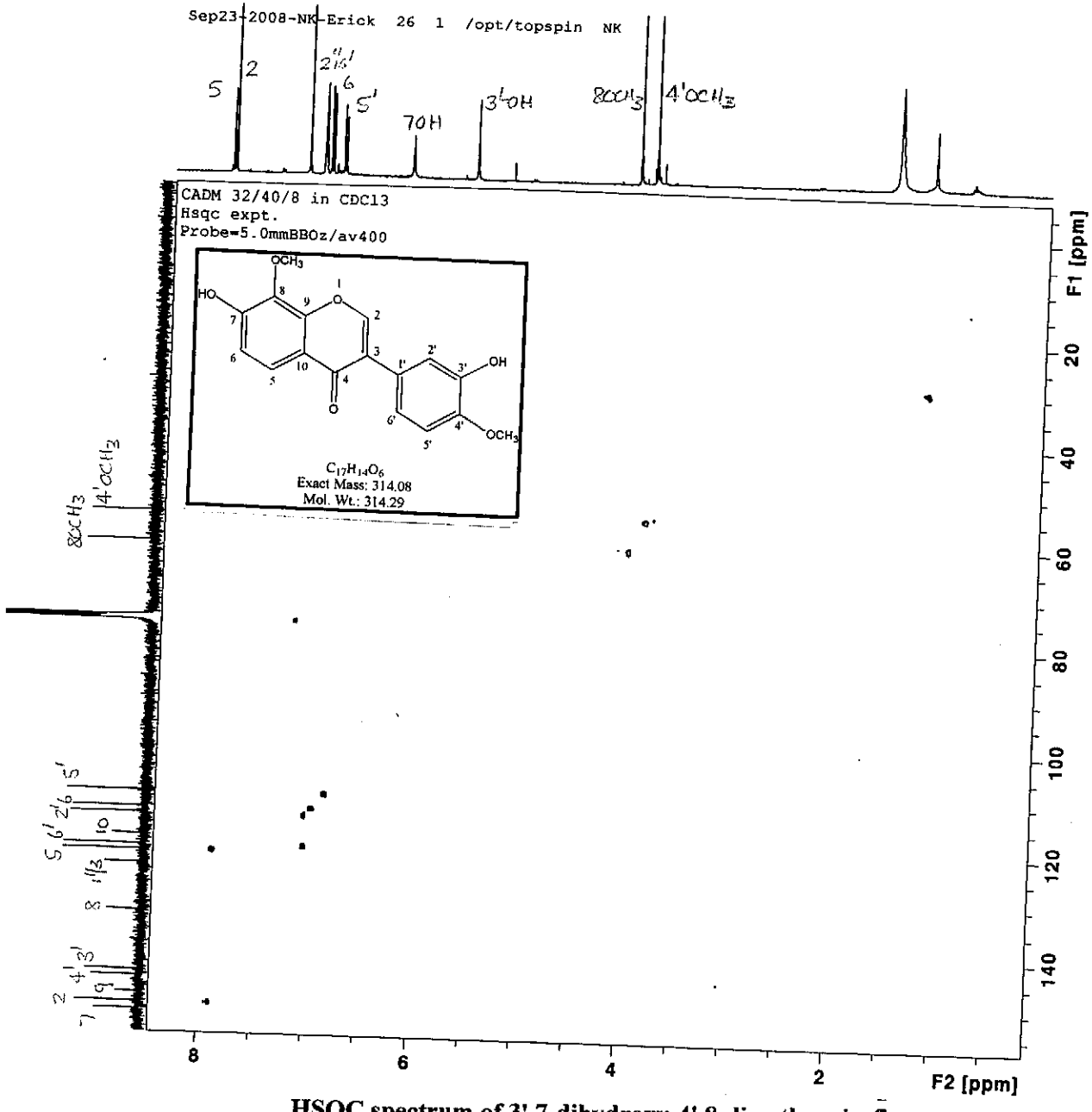
DEPT spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone BS



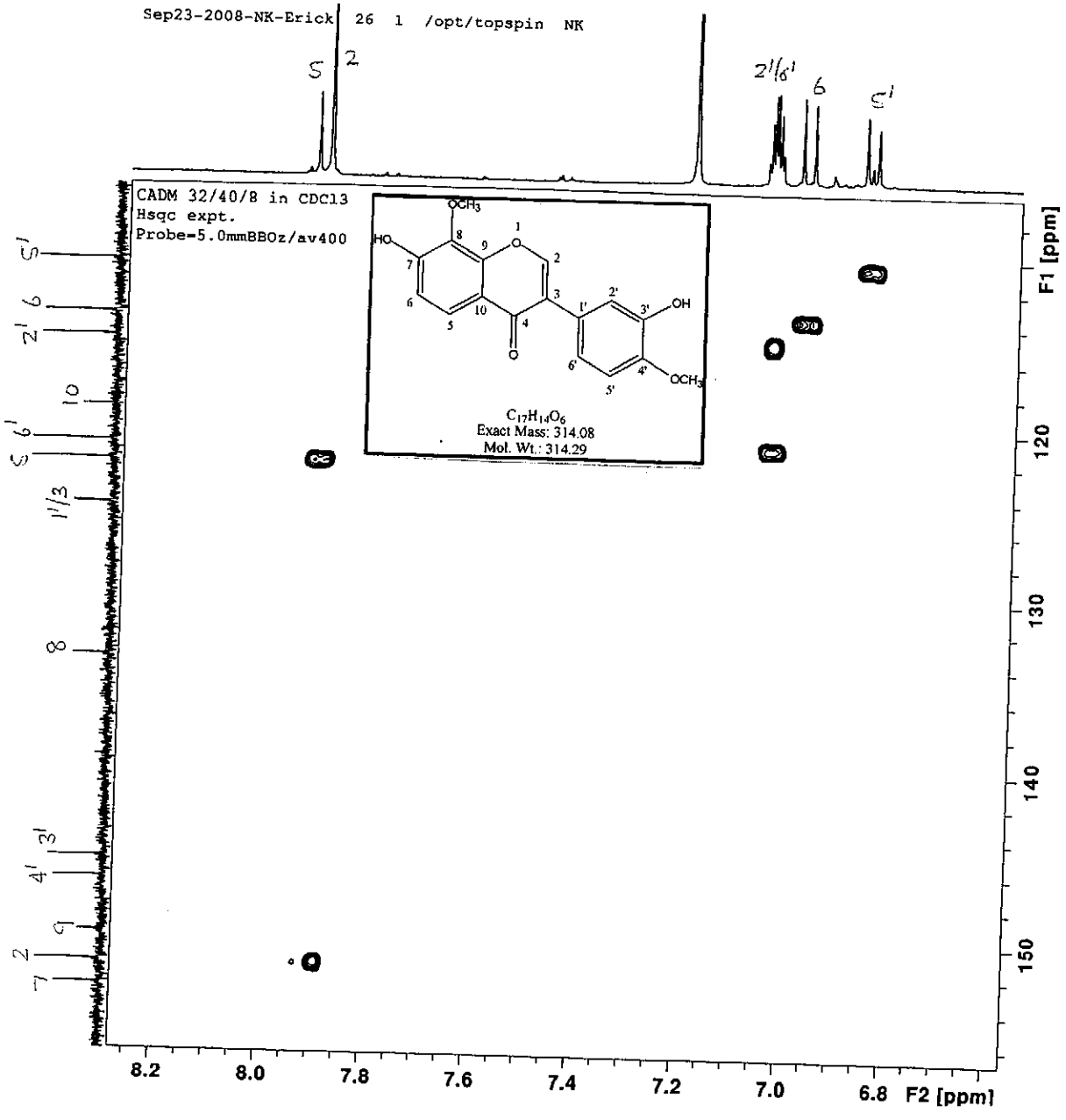
COSY spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone B5



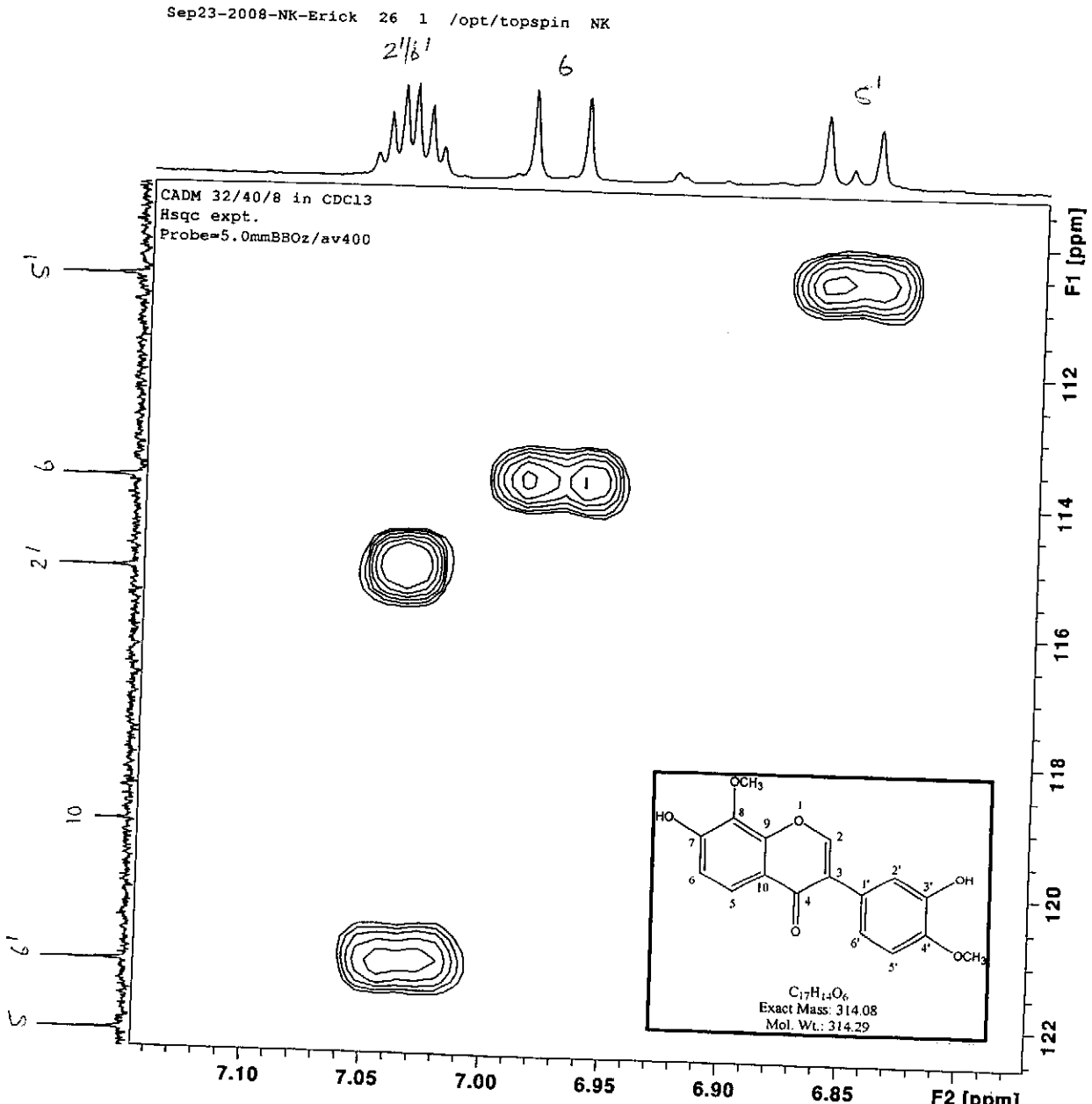
Expanded COSY spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone



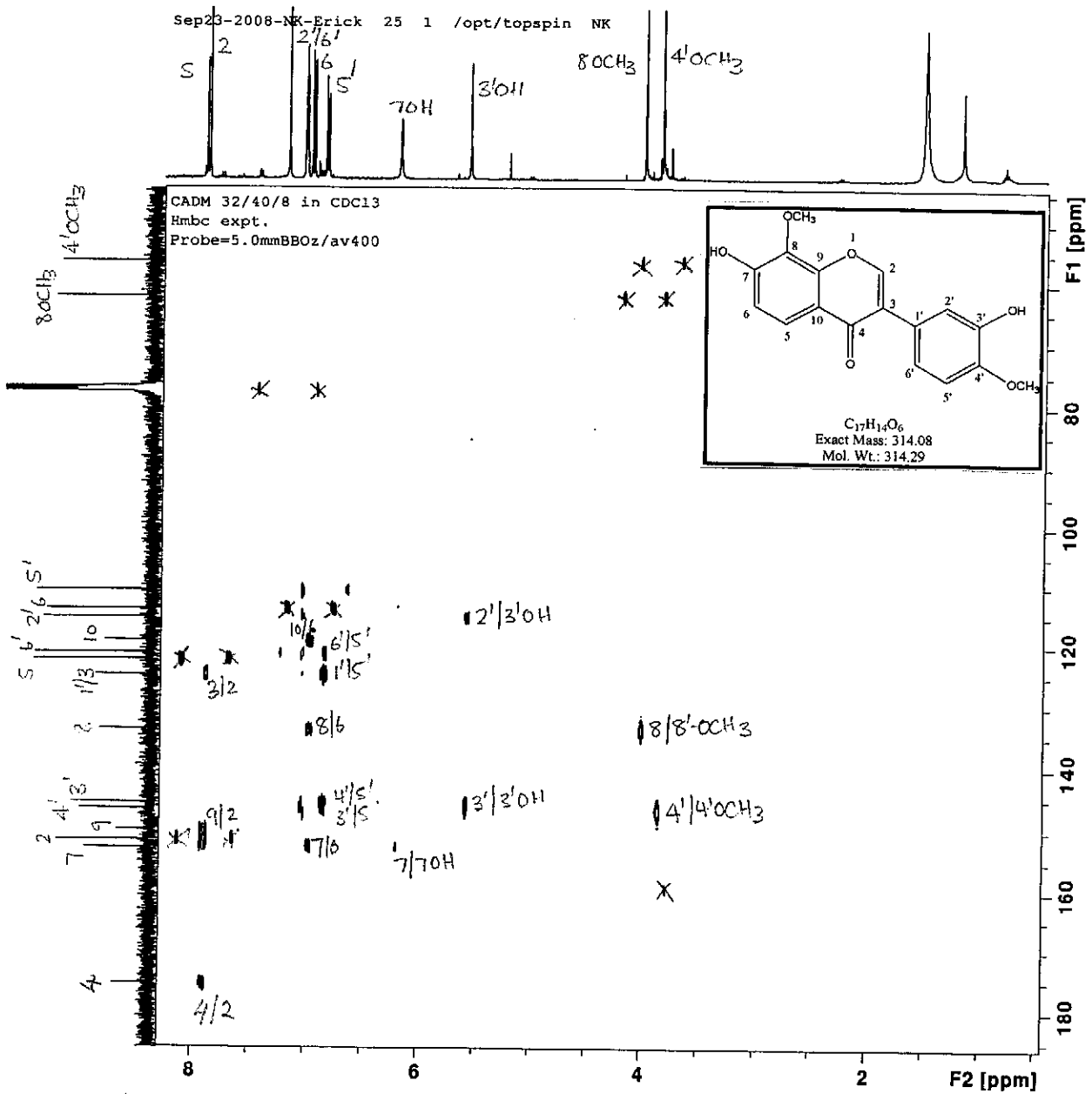
HSQC spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone 85



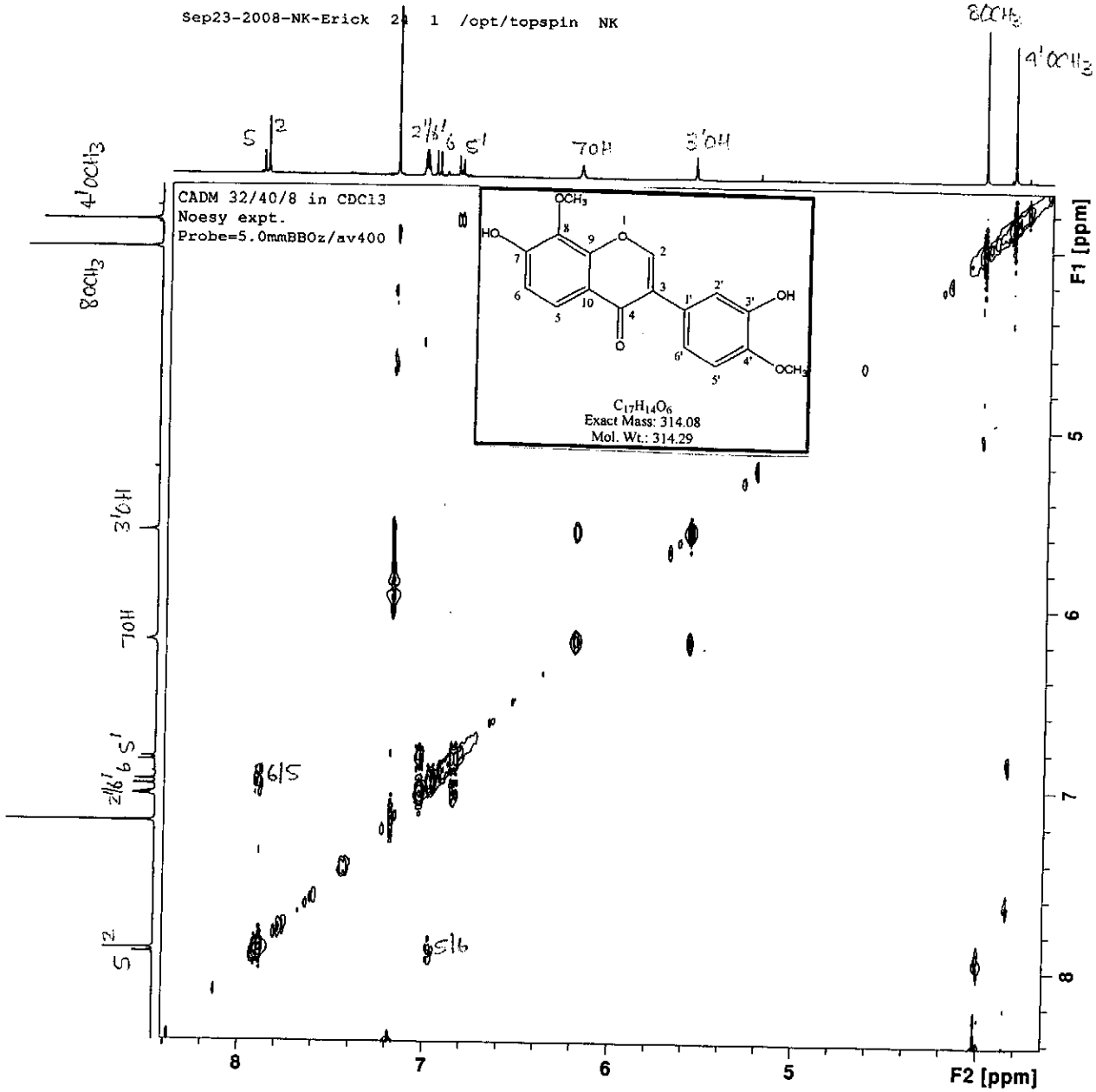
Expanded HSQC spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone 85



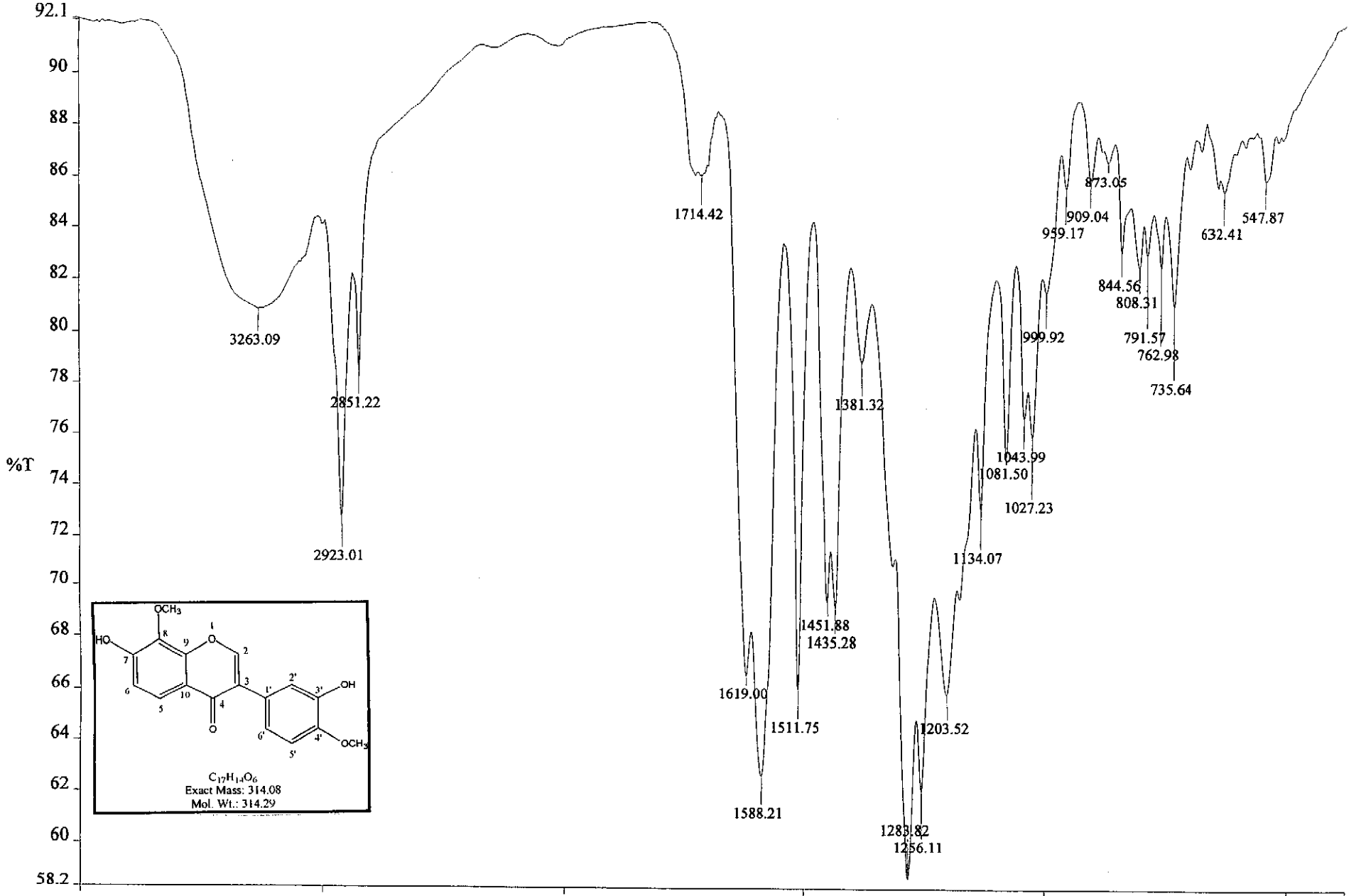
Expanded HSQC spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone BS



HMBC spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone

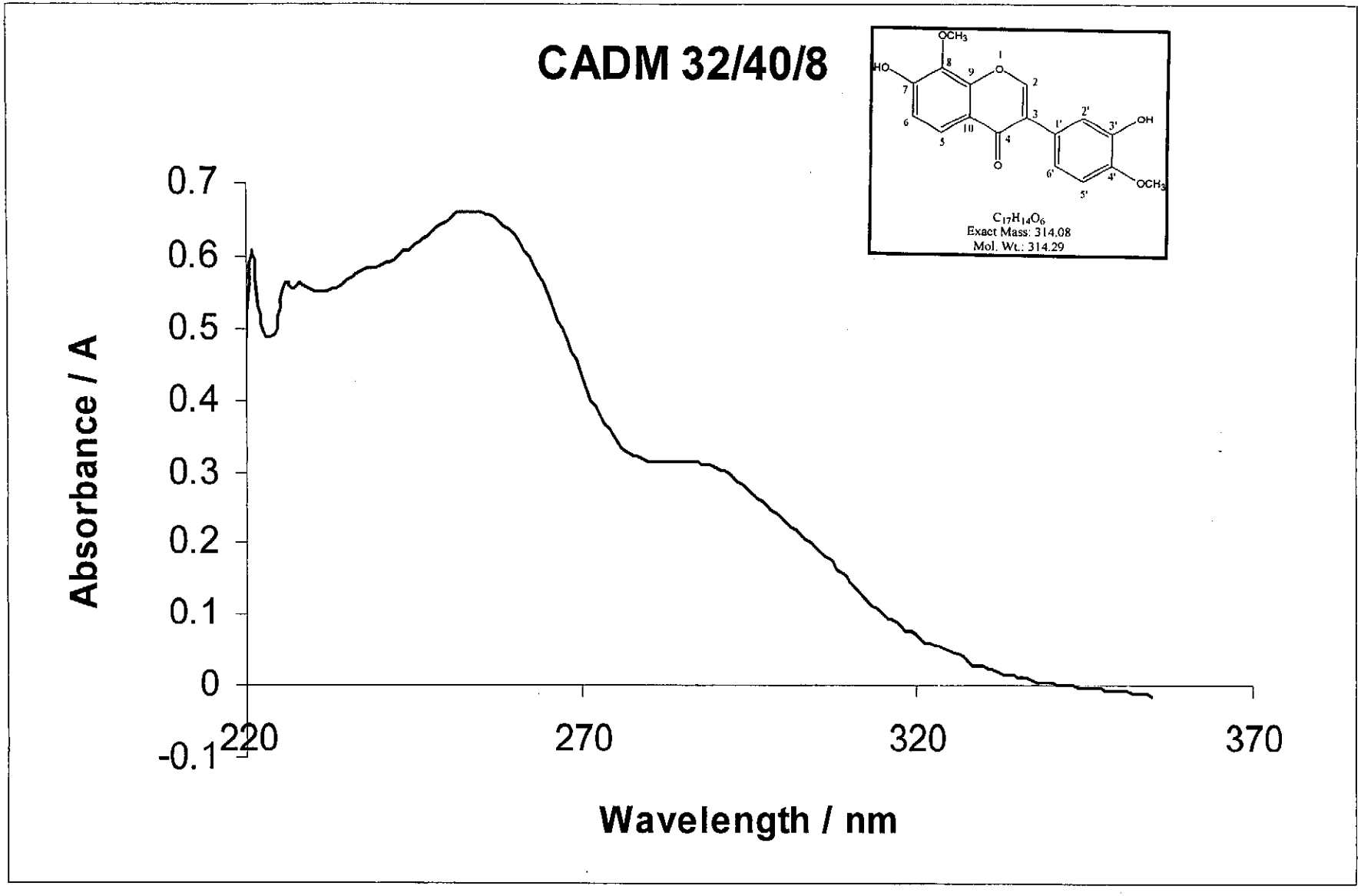


NOESY spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone



IR spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone BS

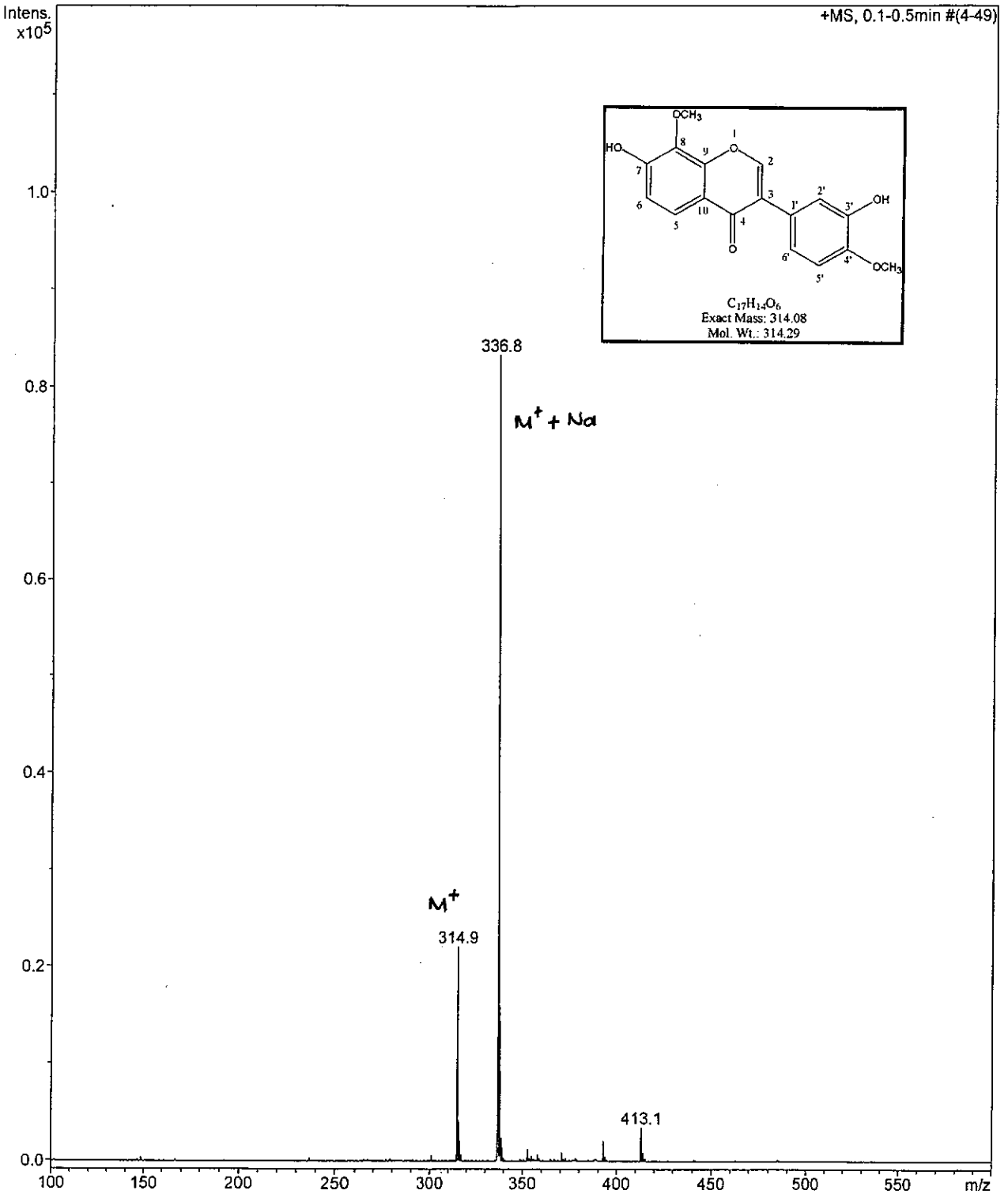
c:\pel_data\spectra\korir\cadm 32-40-E



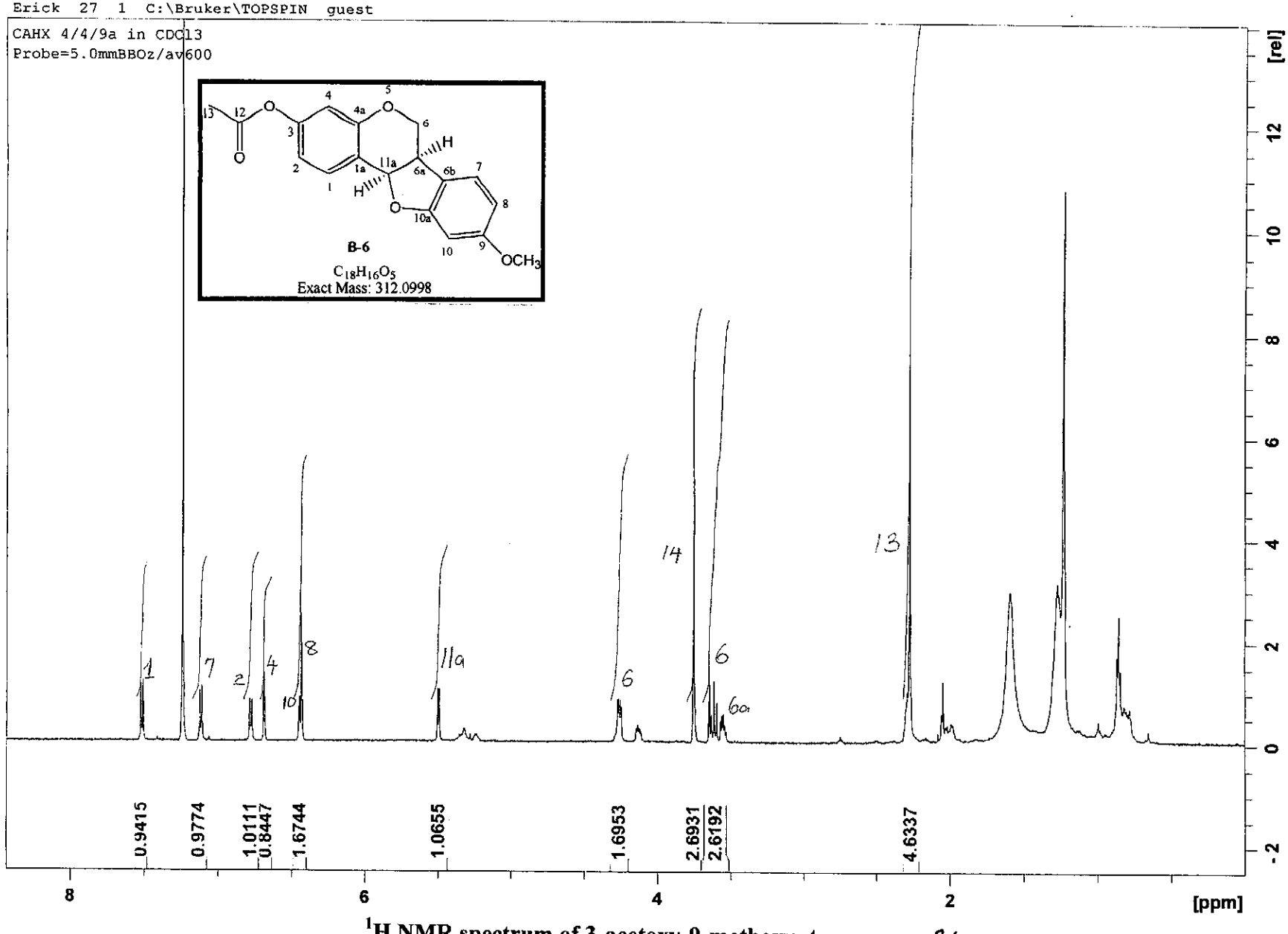
UV spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone 85

Display Report - All Windows Selected Analysis

Analysis Name: ERICK4.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 10/12/2009 08:33:42 PM
Method: FIA.M **Operator:** Administrator **Acq. Date:** 10/12/2009 8:28:20 PM
Sample Name: ERICK4
Analysis Info: CADM32/40/8

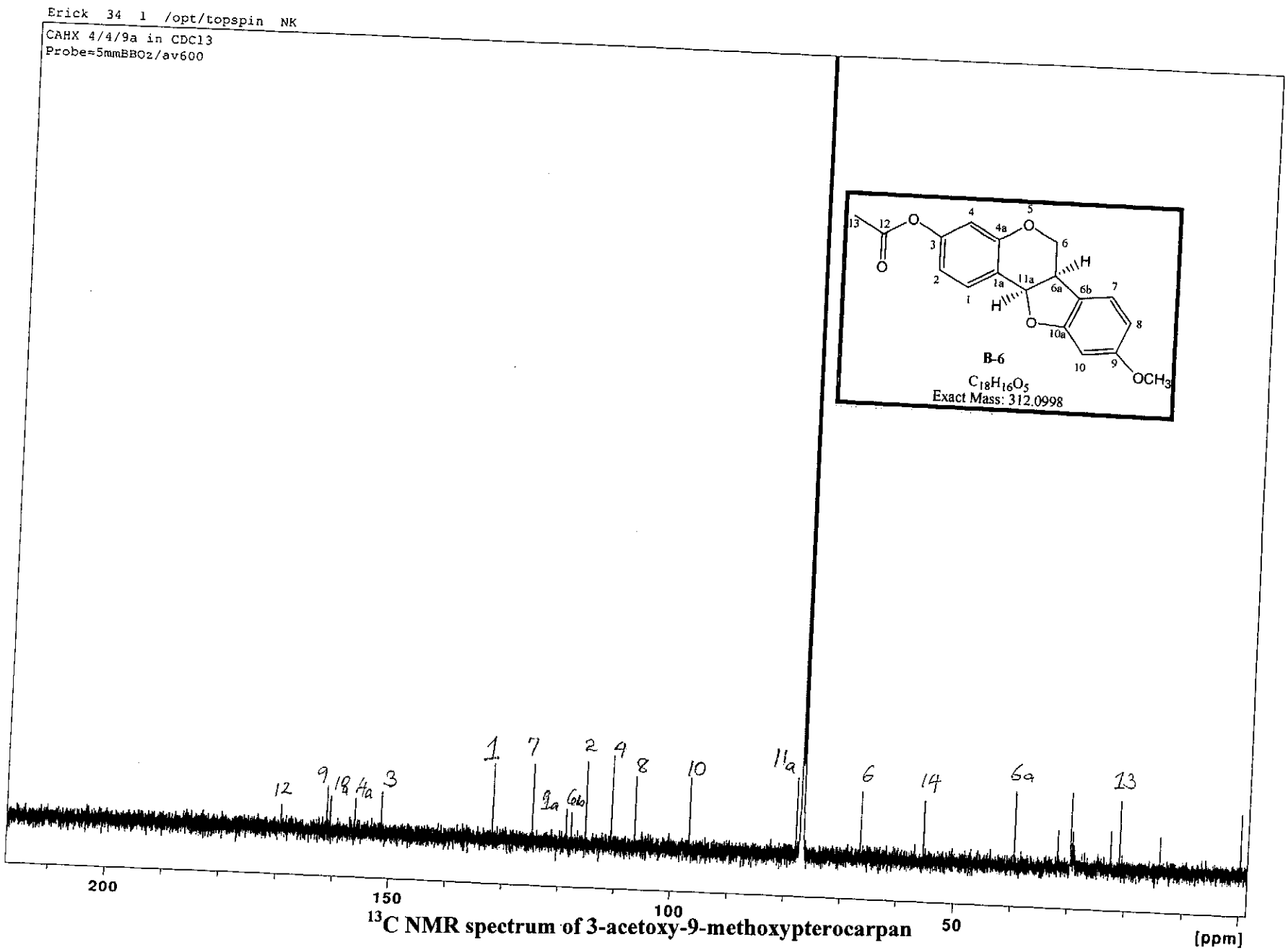


Mass spectrum of 3',7-dihydroxy-4',8-dimethoxyisoflavone BS



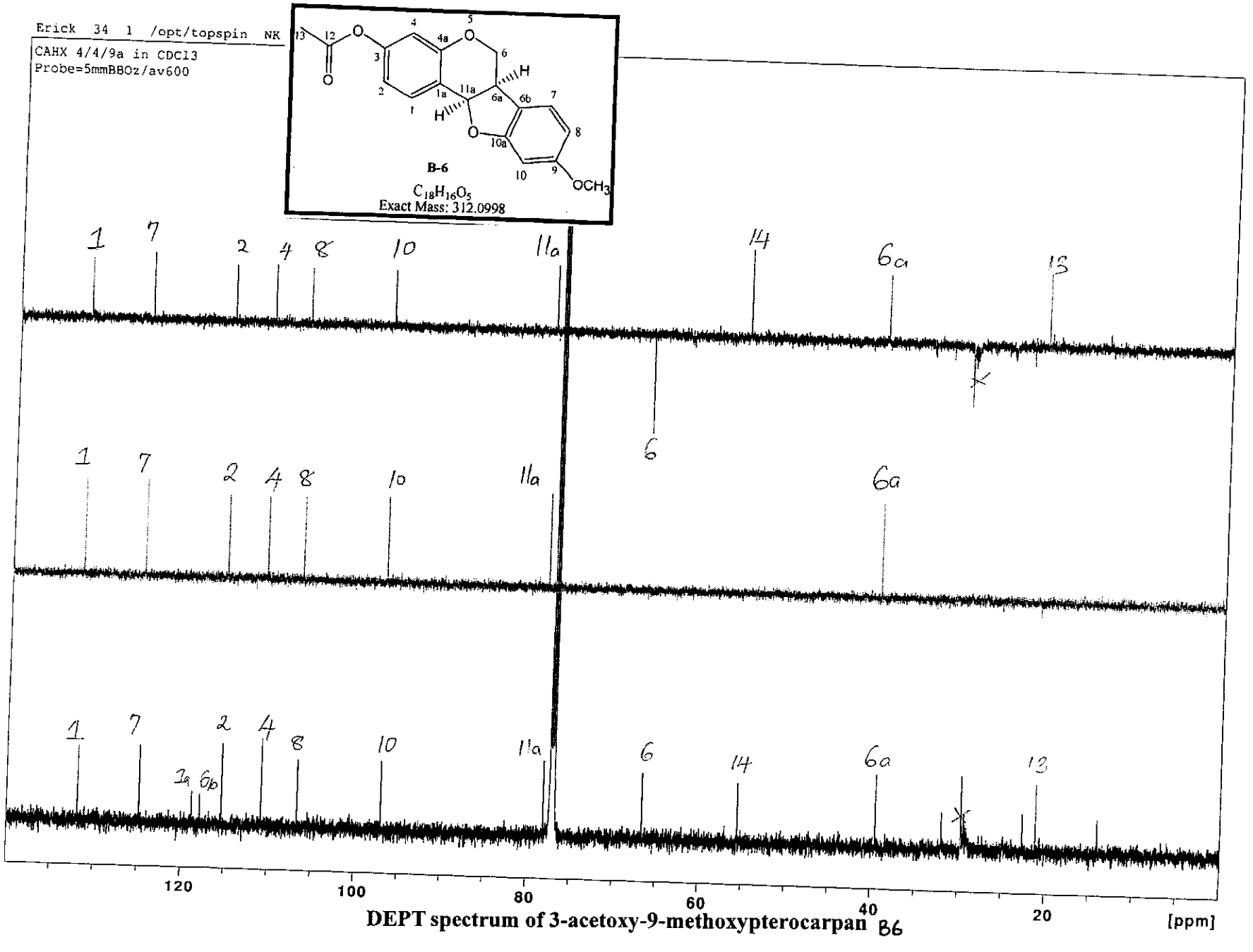
^{1}H NMR spectrum of 3-acetoxy-9-methoxypterocarpan B6

Peak	ν(F1) [ppm]	Intensity [abs]	Annotation
1	7.5228	317449.48	
2	7.5089	316992.25	
3	7.2401	4647398.34	
4	7.1238	294025.36	
5	7.1108	343159.50	
6	6.7858	263433.34	
7	6.7823	211182.36	
8	6.7720	259879.03	
9	6.7685	194745.98	
10	6.6892	432671.61	
11	6.6859	355958.27	
12	6.4507	223372.64	
13	6.4475	246686.27	
14	6.4337	723102.02	
15	5.5035	328392.64	
16	5.4922	328742.16	
17	5.3190	95143.84	
18	4.2694	247217.20	
19	4.2616	278384.16	
20	4.2517	230846.08	
21	4.2436	226495.33	
22	4.1433	98681.41	
23	4.1342	115468.17	
24	4.1247	105744.84	
25	4.1147	94848.95	
26	3.7513	1896734.80	
27	3.6446	392855.61	
28	3.6297	165976.67	
29	3.6116	383047.30	
30	3.5934	239119.73	
31	3.5679	142273.56	
32	3.5579	171090.30	
33	3.5489	185910.94	
34	3.5415	116922.78	
35	3.5308	81810.95	
36	2.2902	319713.86	
37	2.2751	2318616.00	

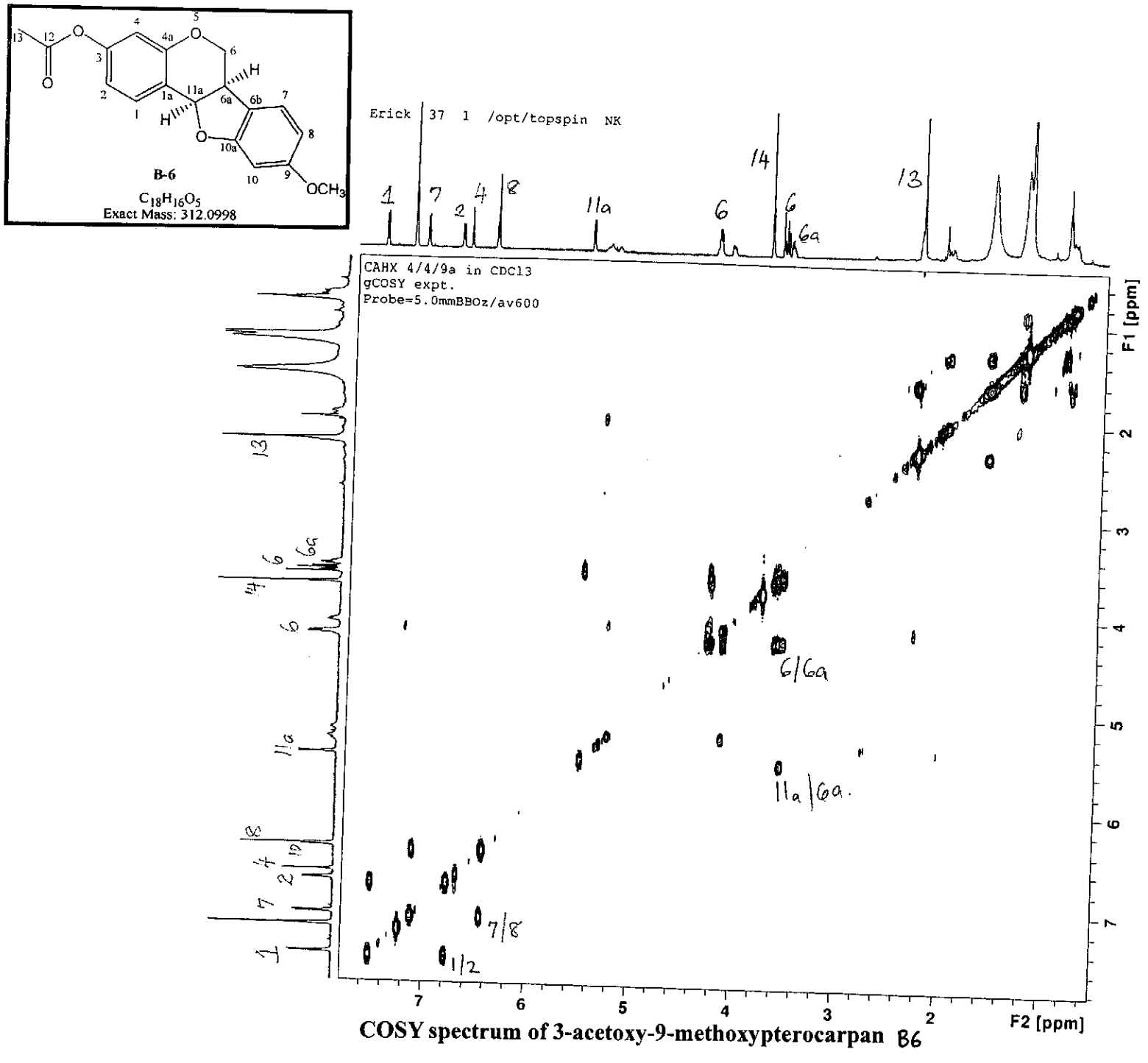


^{13}C NMR spectrum of 3-acetoxy-9-methoxypterocarpan

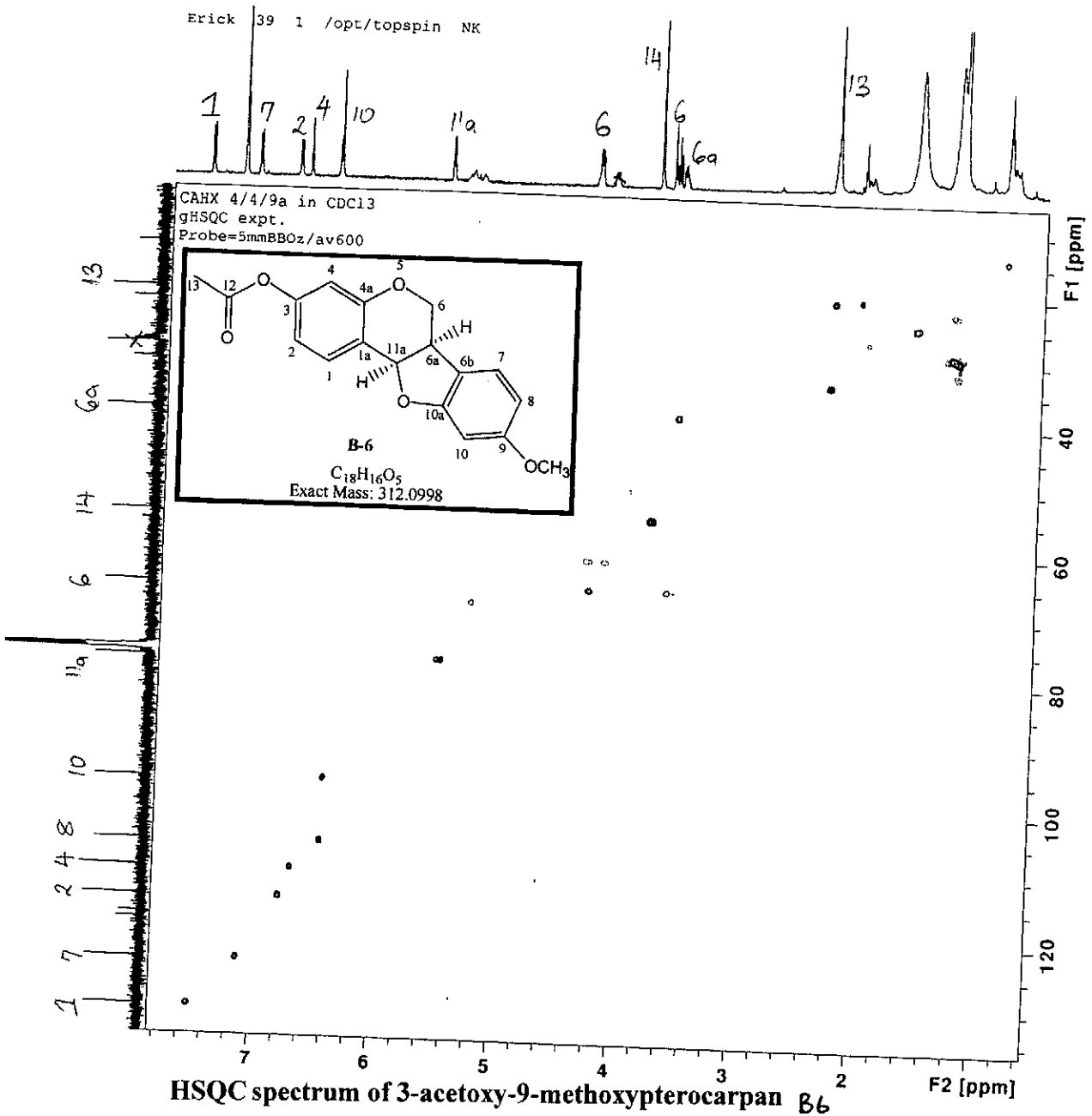
Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity	Annotation
1	169.2130	25533.4405	0.06	
2	161.2166	24326.8216	0.10	
3	160.5883	24232.0141	0.08	
4	156.2523	23577.7322	0.08	
5	151.6276	22879.8869	0.10	
6	131.8212	19891.1949	0.17	
7	124.7840	18829.3147	0.17	
8	118.7800	17923.3396	0.08	
9	117.8382	17781.2264	0.07	
10	115.3182	17400.9703	0.18	
11	110.7533	16712.1486	0.20	
12	106.5799	16082.4023	0.16	
13	96.9284	14626.0366	0.16	
14	78.0562	11778.3110	0.18	
15	77.2234	11652.6454	15.00	
16	77.0119	11620.7311	14.99	
17	76.8002	11588.7865	14.86	
18	66.6158	10052.0088	0.16	
19	55.5232	8378.1880	0.14	
20	39.5389	5966.2328	0.17	
21	31.9279	4817.7689	0.08	
22	29.7028	4482.0119	0.16	
23	29.3624	4430.6471	0.08	
24	29.0899	4389.5282	0.05	
25	22.6946	3424.5077	0.09	
26	21.1229	3187.3456	0.15	
27	14.1204	2130.7015	0.08	
28	-0.0050	-0.7545	0.13	



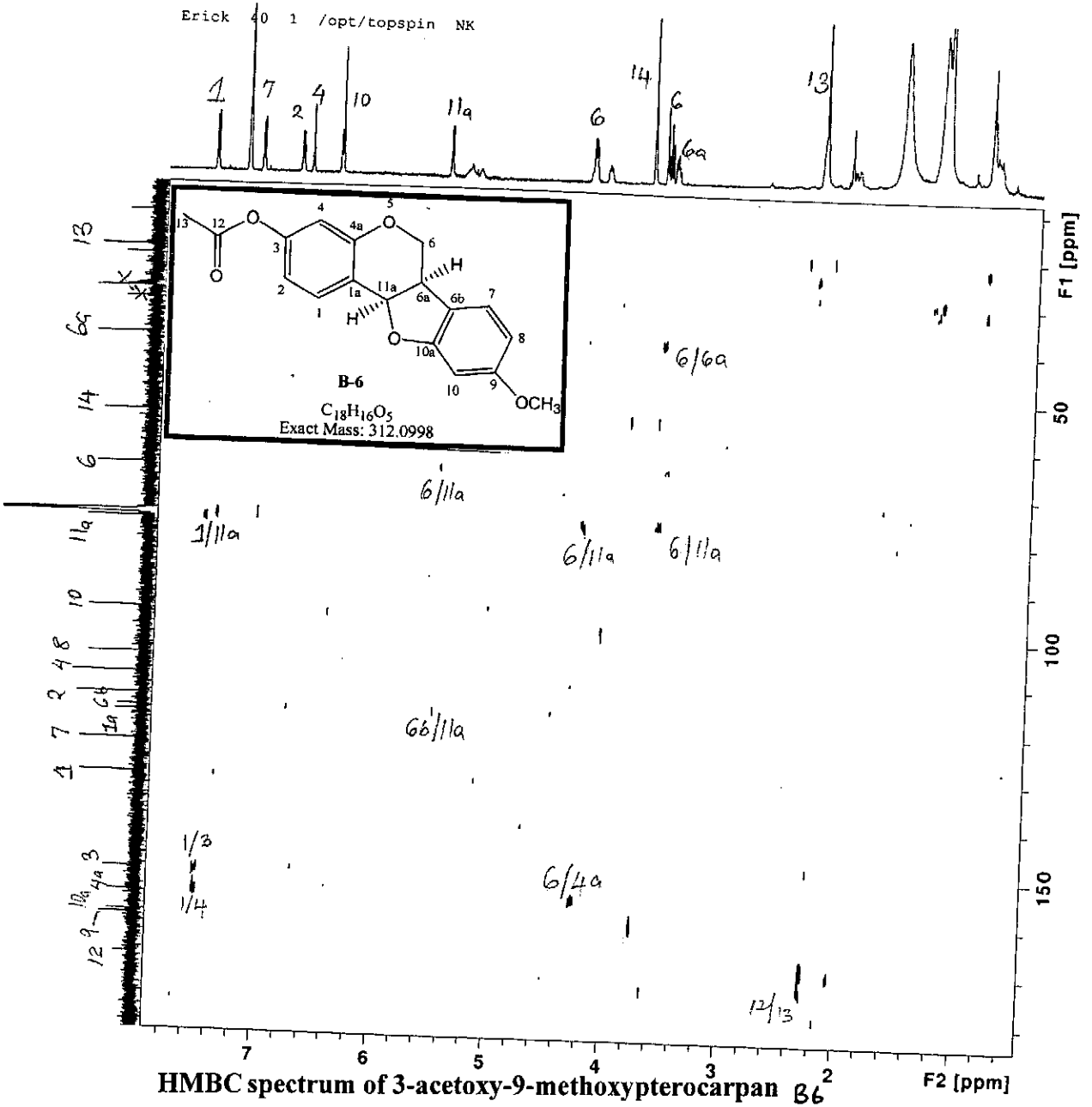
DEPT spectrum of 3-acetoxy-9-methoxypterocarpan B6



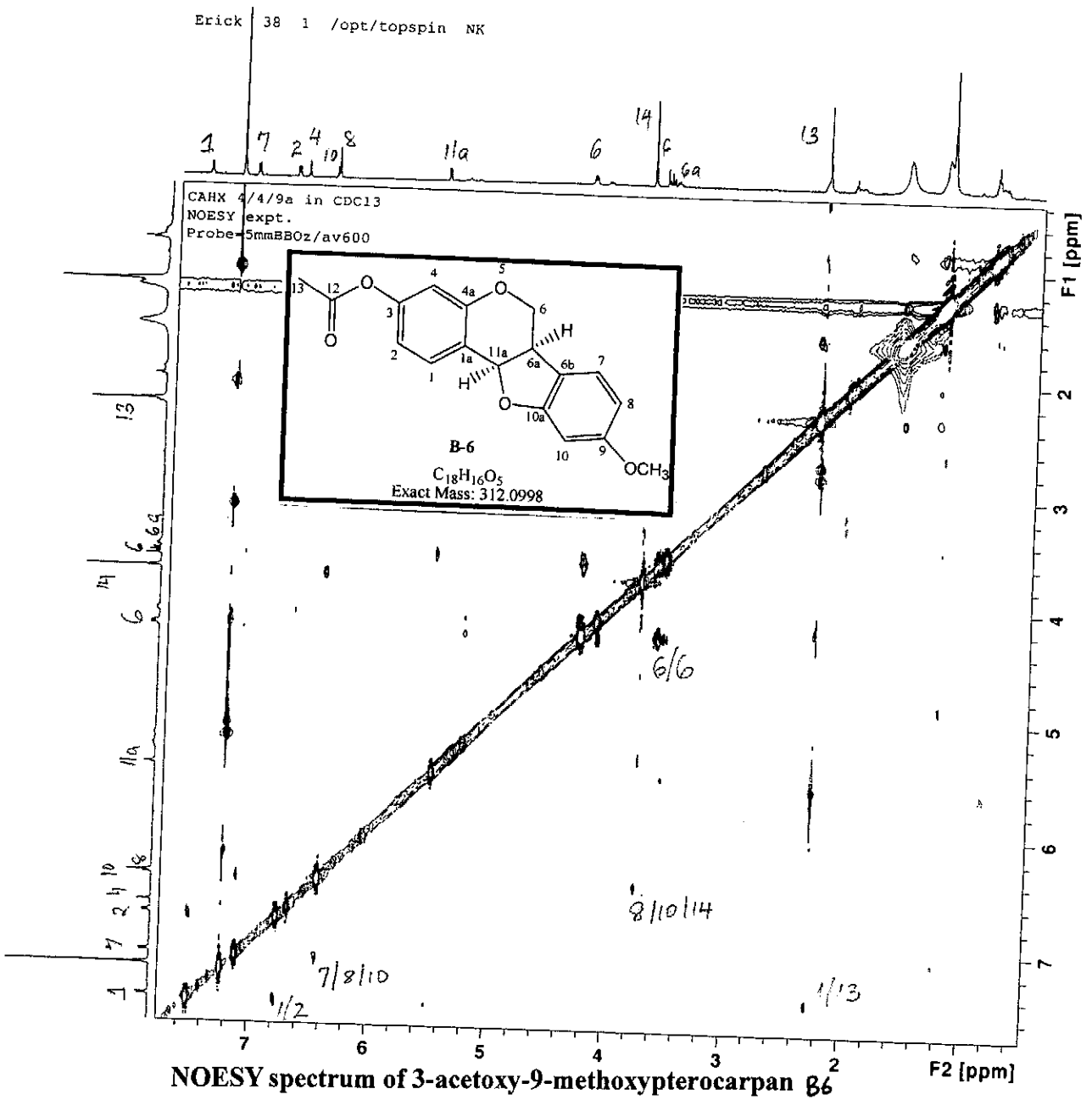
COSY spectrum of 3-acetoxy-9-methoxypterocarpan B6



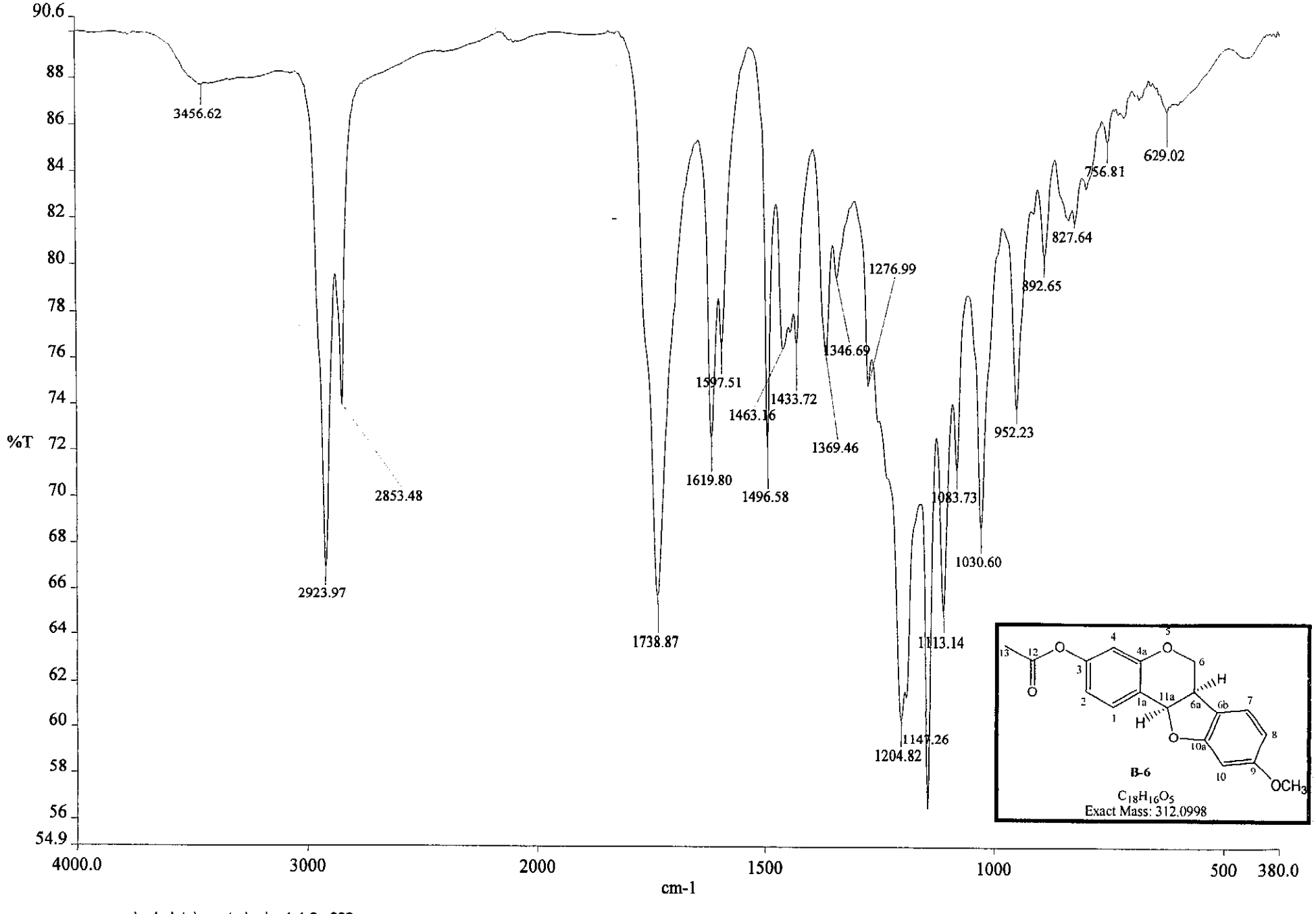
HSQC spectrum of 3-acetoxy-9-methoxypterocarpan B6



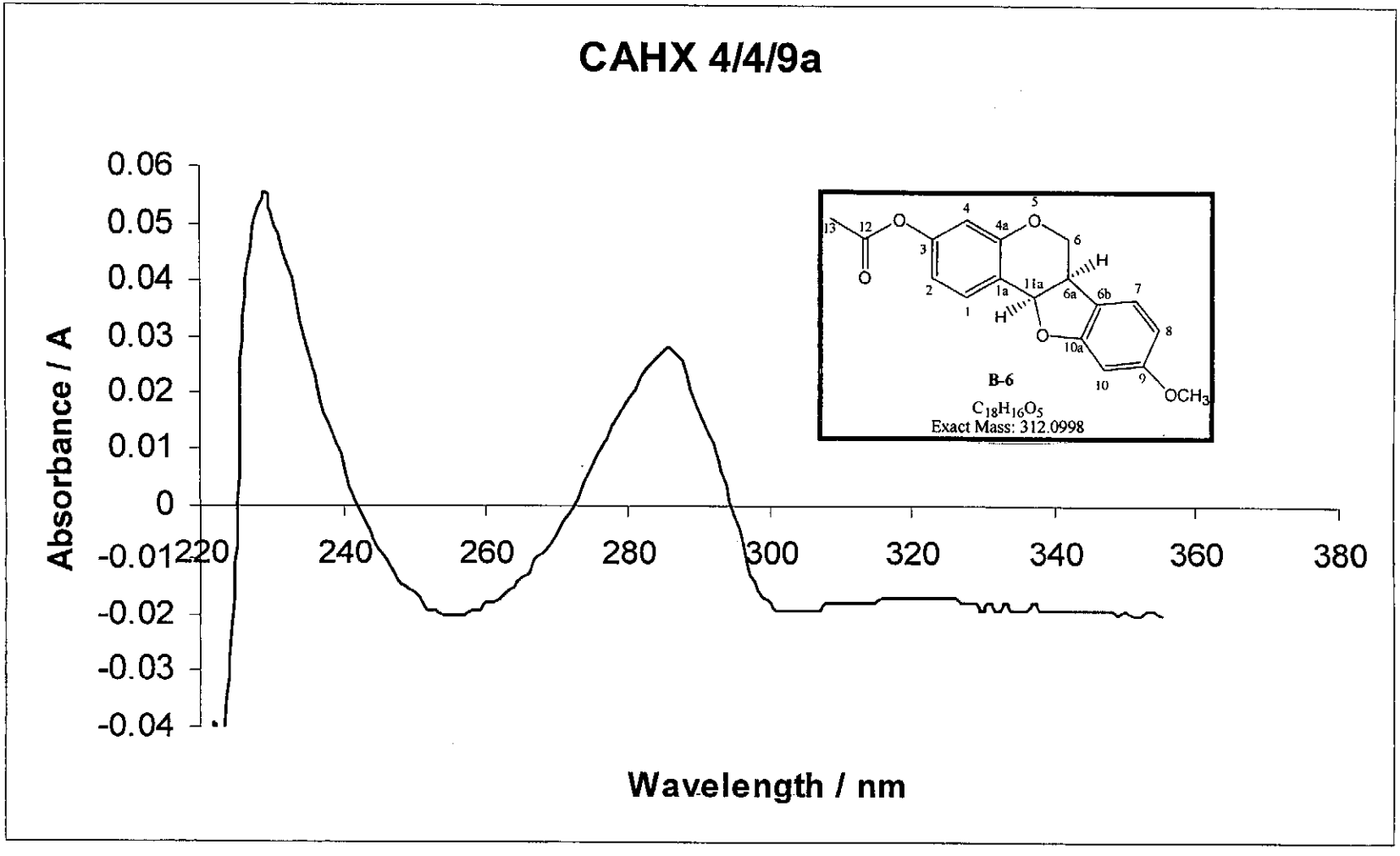
HMBC spectrum of 3-acetoxy-9-methoxypterocarpan B6



NOESY spectrum of 3-acetoxy-9-methoxypterocarpan B6

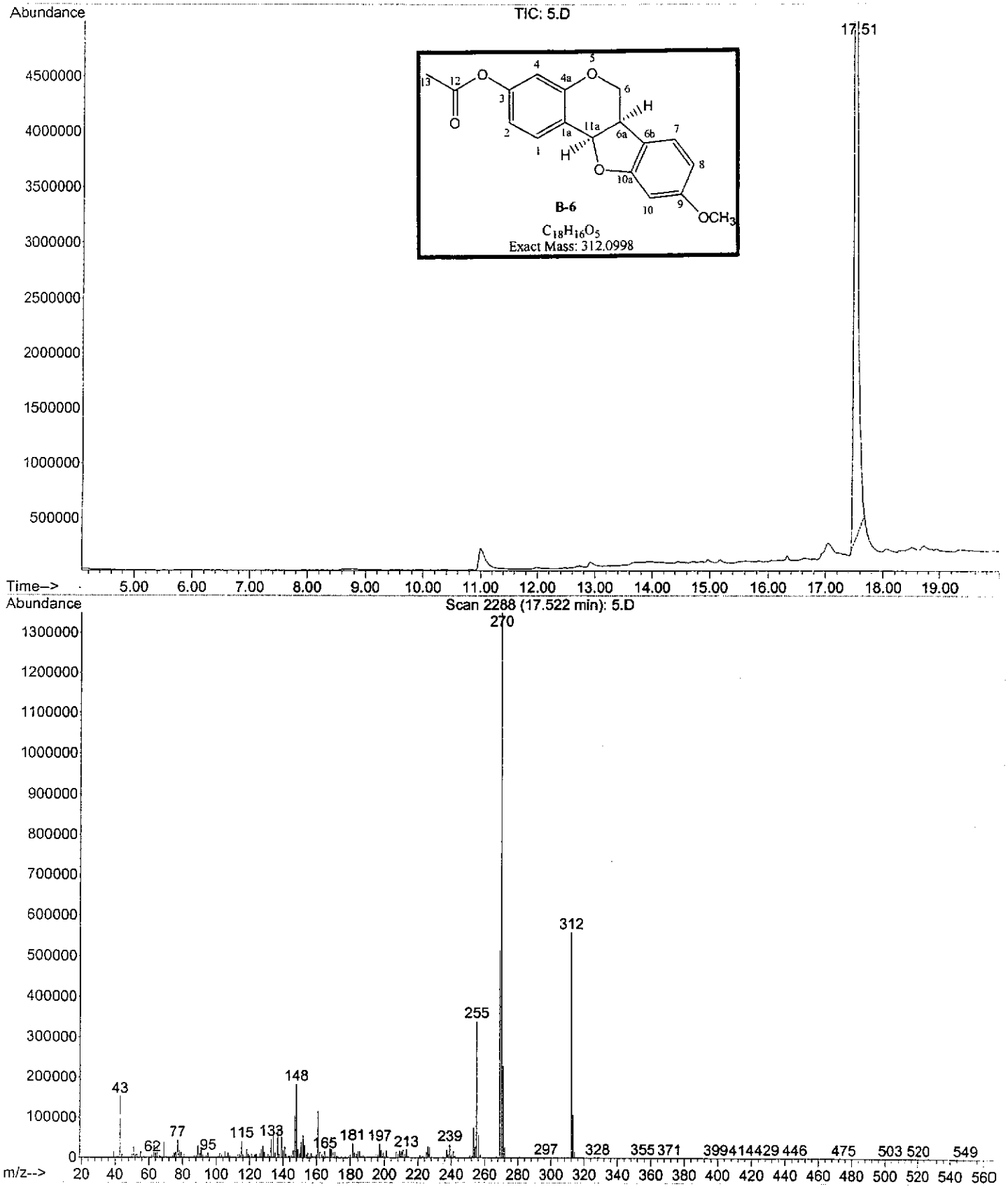


IR spectrum of 3-acetoxy-9-methoxypterocarpan B6

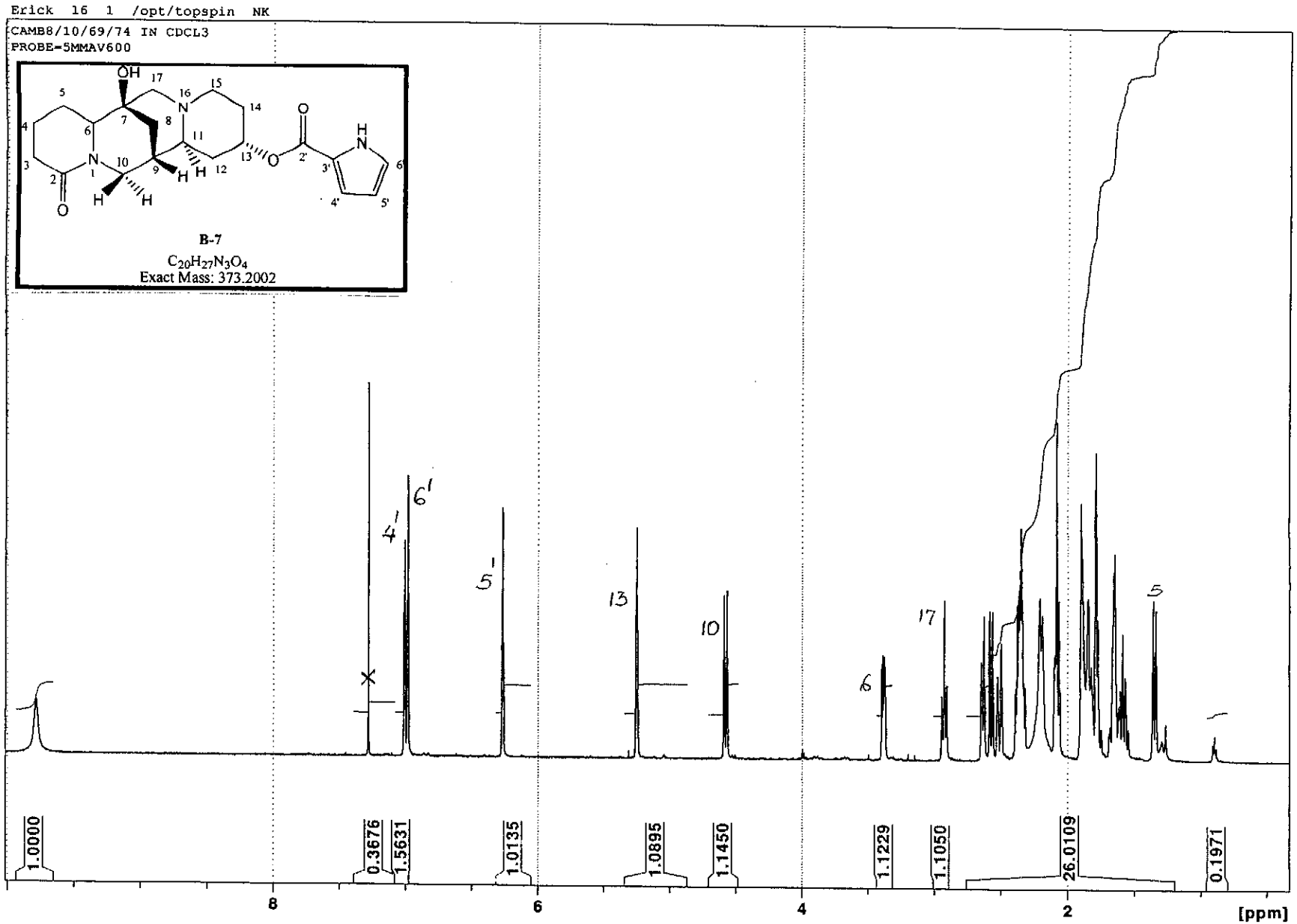


UV spectrum of 3-acetoxy-9-methoxypterocarpan B6

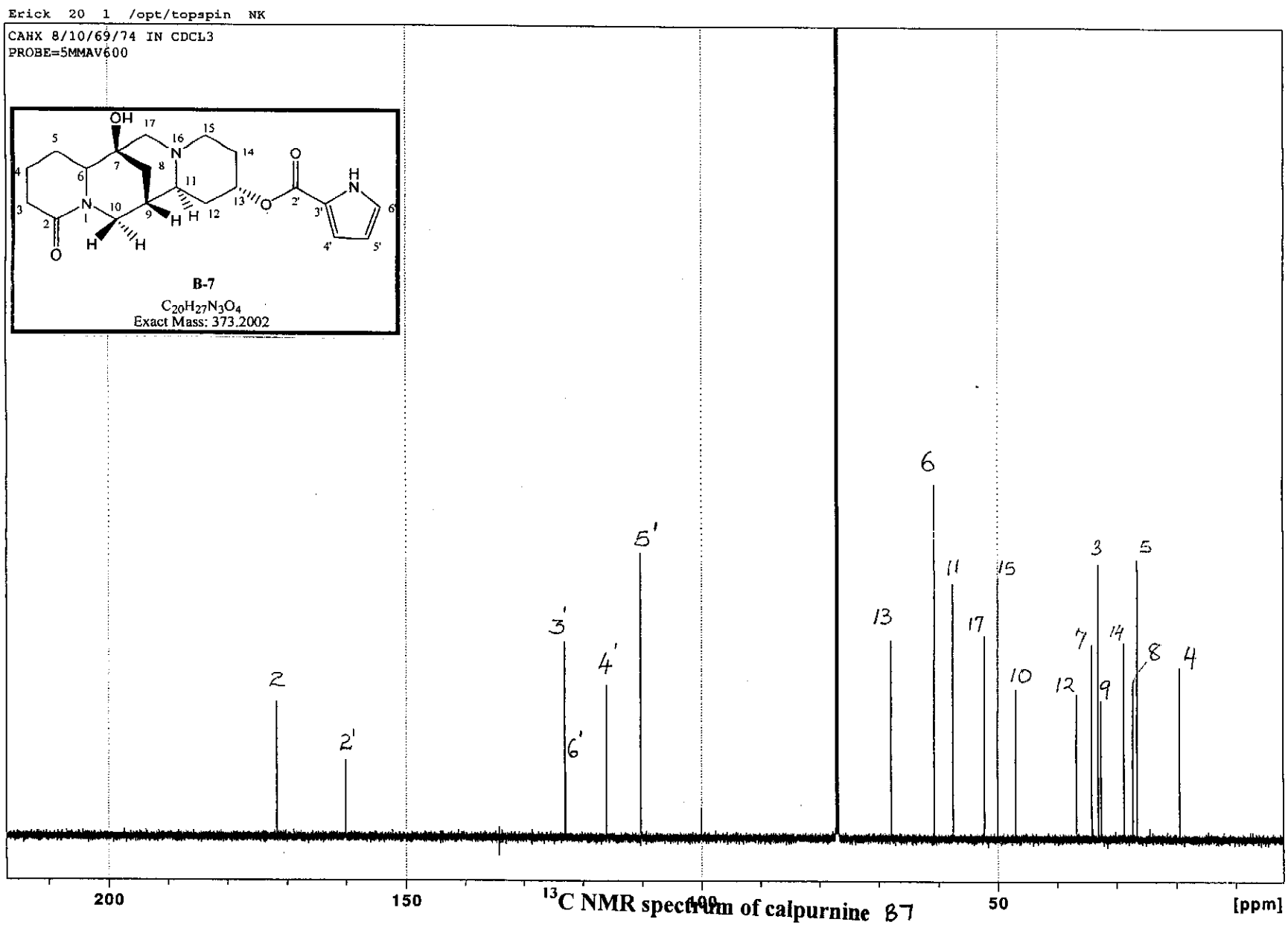
File : D:\ERICK2009\5.D
Operator : ERICK
Acquired : 4 Sep 2009 21:10 using AcqMethod ERICK2
Instrument : Instrumen
Sample Name: 040909 RERUN 3ULCHANGED OVEN TEMP
Misc Info : CAMX 4/4/99
Vial Number: 1



Mass spectrum of 3-acetoxy-9-methoxypterocarpan B6



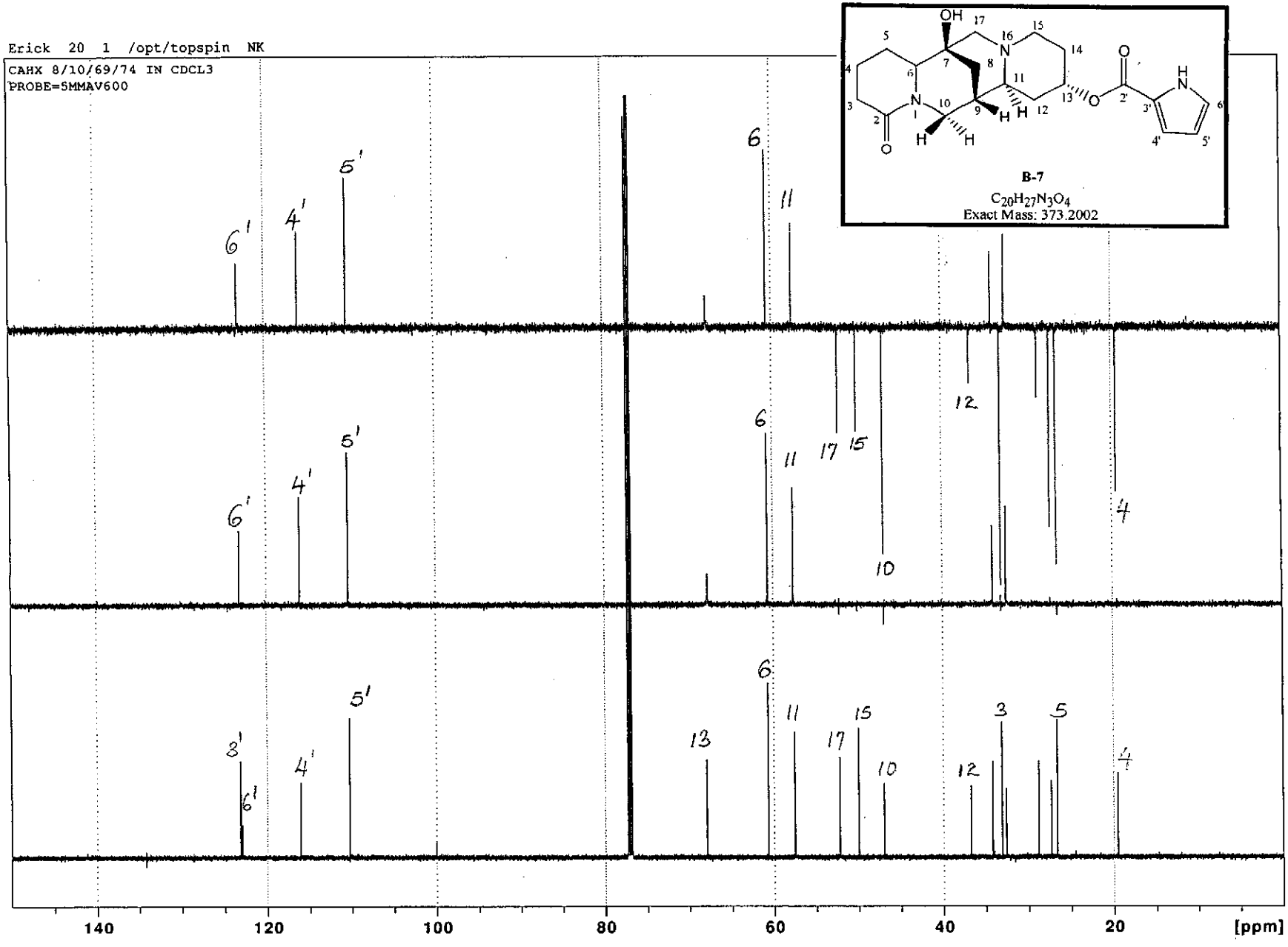
^{1}H NMR spectrum of calpurnine B7



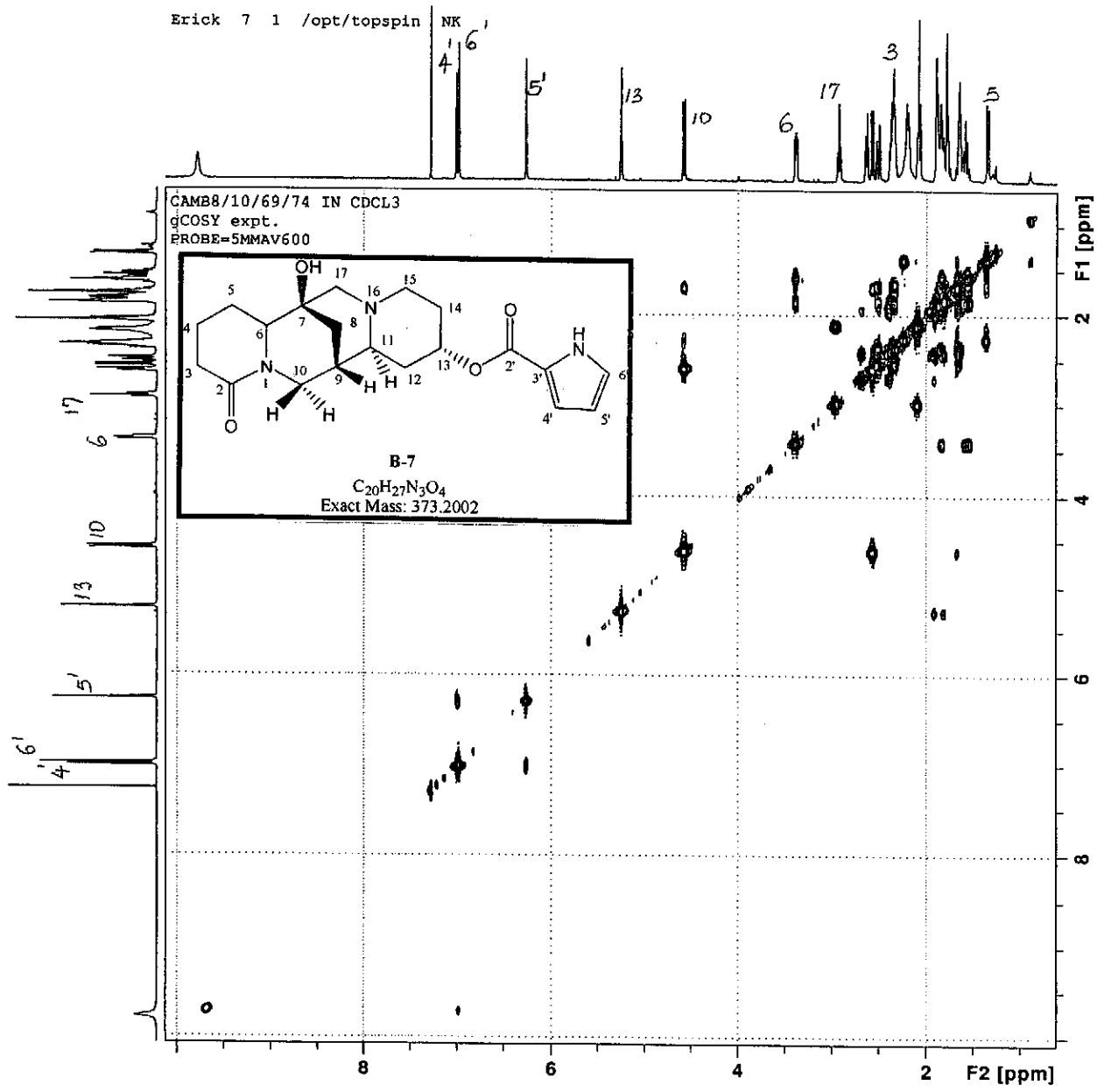
^{13}C NMR spectrum of calpurnine B7

Peak	ν(F1) [ppm]	ν(F1) [Hz]	Intensity	Annotation
1	171.7321	25913.5607	1.82	
2	160.1001	24158.3470	0.76	
3	123.1130	18577.1688	1.91	
4	122.9259	18548.9363	0.66	
5	116.0268	17507.8947	1.52	
6	110.2568	16637.2291	2.76	
7	99.9964	15088.9833	0.29	
8	77.2523	11657.0063	14.60	
9	77.0406	11625.0618	15.00	
10	76.8289	11593.1172	14.96	
11	67.9457	10252.6884	1.93	
12	60.6830	9156.7774	3.43	
13	57.5401	8682.5286	2.47	
14	52.2161	7879.1622	1.97	
15	49.9483	7536.9921	2.63	
16	46.9565	7085.5135	1.51	
17	36.7361	5543.3035	0.44	
18	34.1755	5156.9211	1.89	
19	33.0056	4979.9985	2.67	
20	32.5975	4918.8084	1.42	
21	28.7762	4342.4941	1.804	
22	27.2965	4118.9126	1.54	
23	26.5909	4012.4406	2.51	
24	19.4503	2934.9582	1.70	

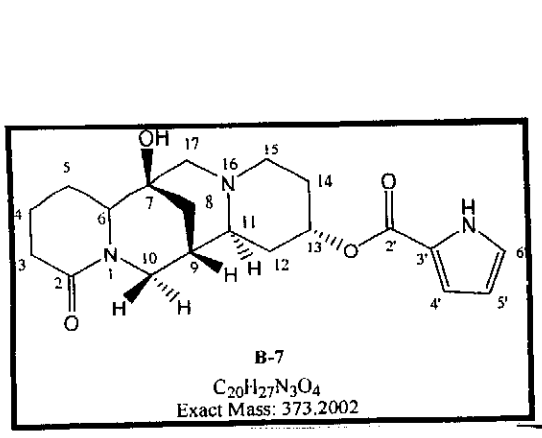
^{13}C NMR spectrum of calpurnine

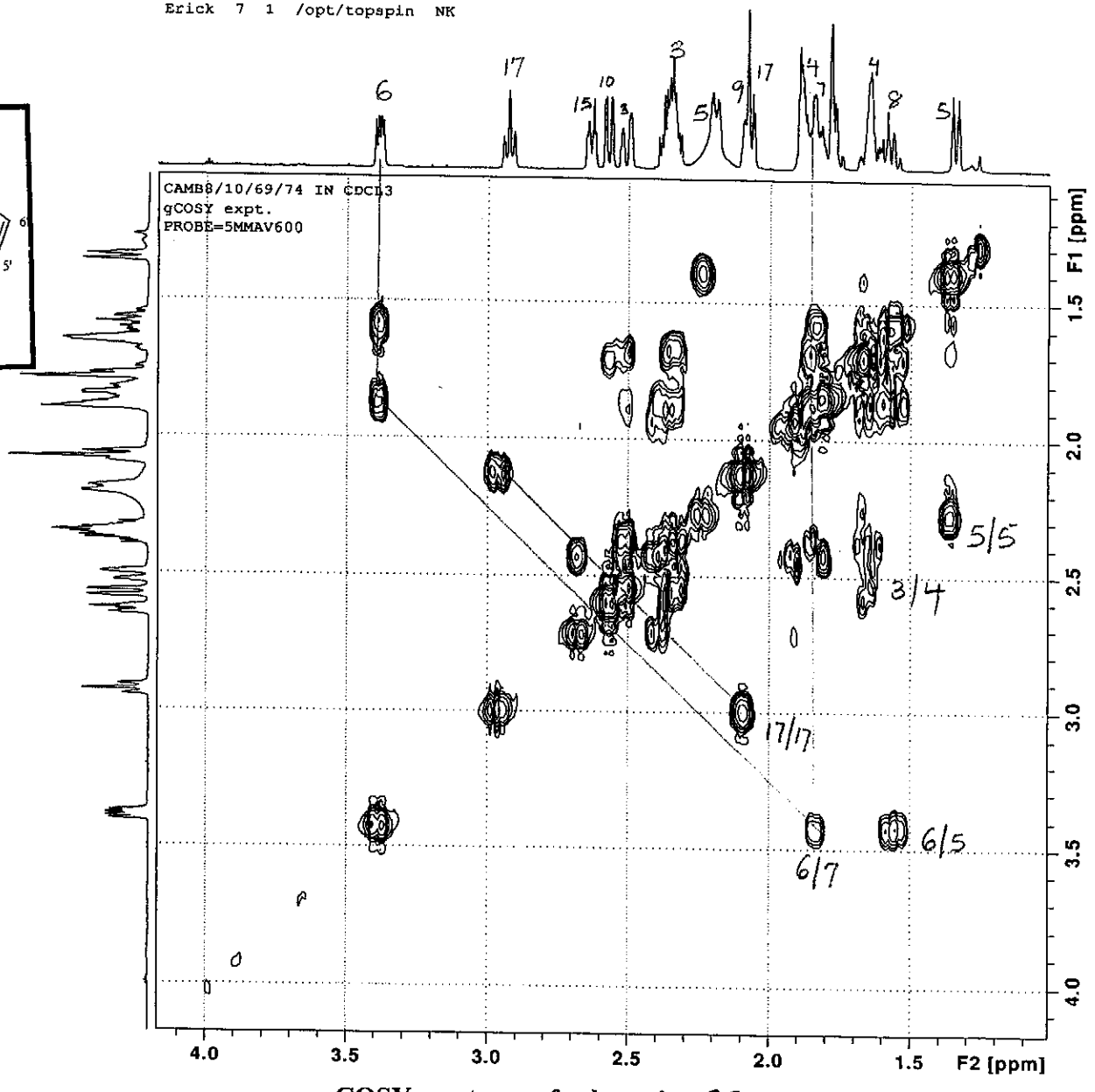


DEPT spectrum of calpurnine B7

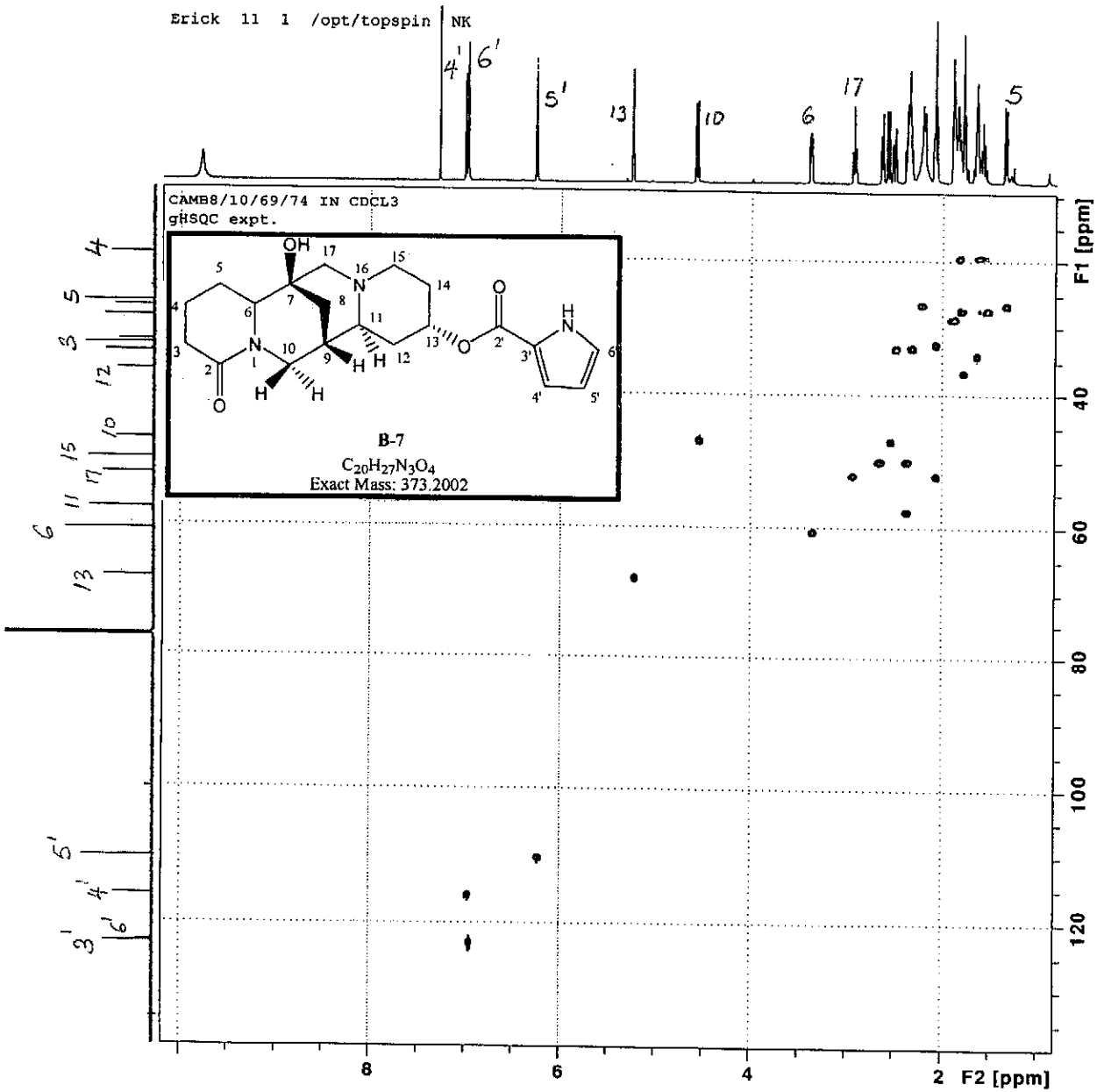


COSY spectrum of calpurnine B-7

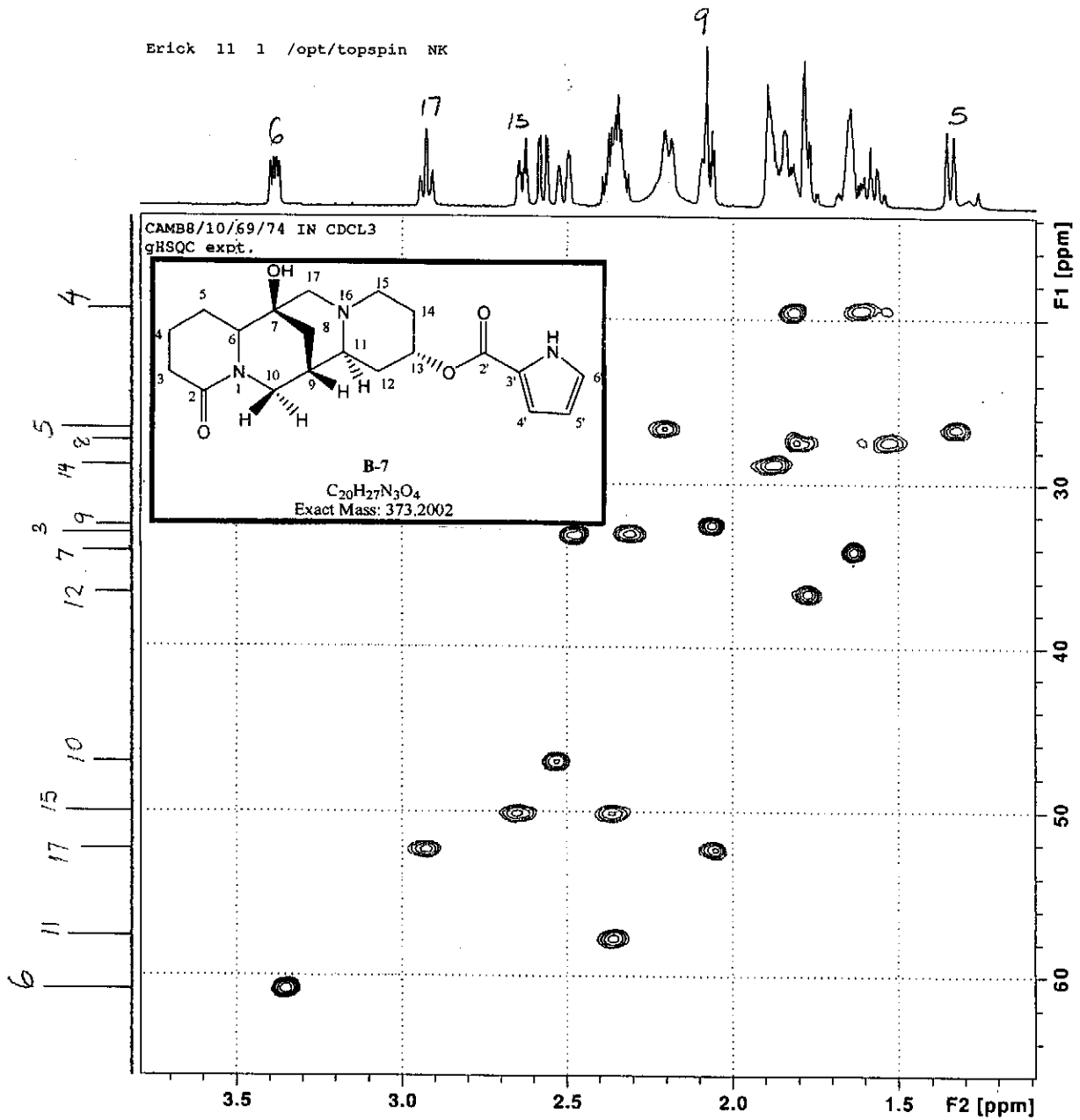




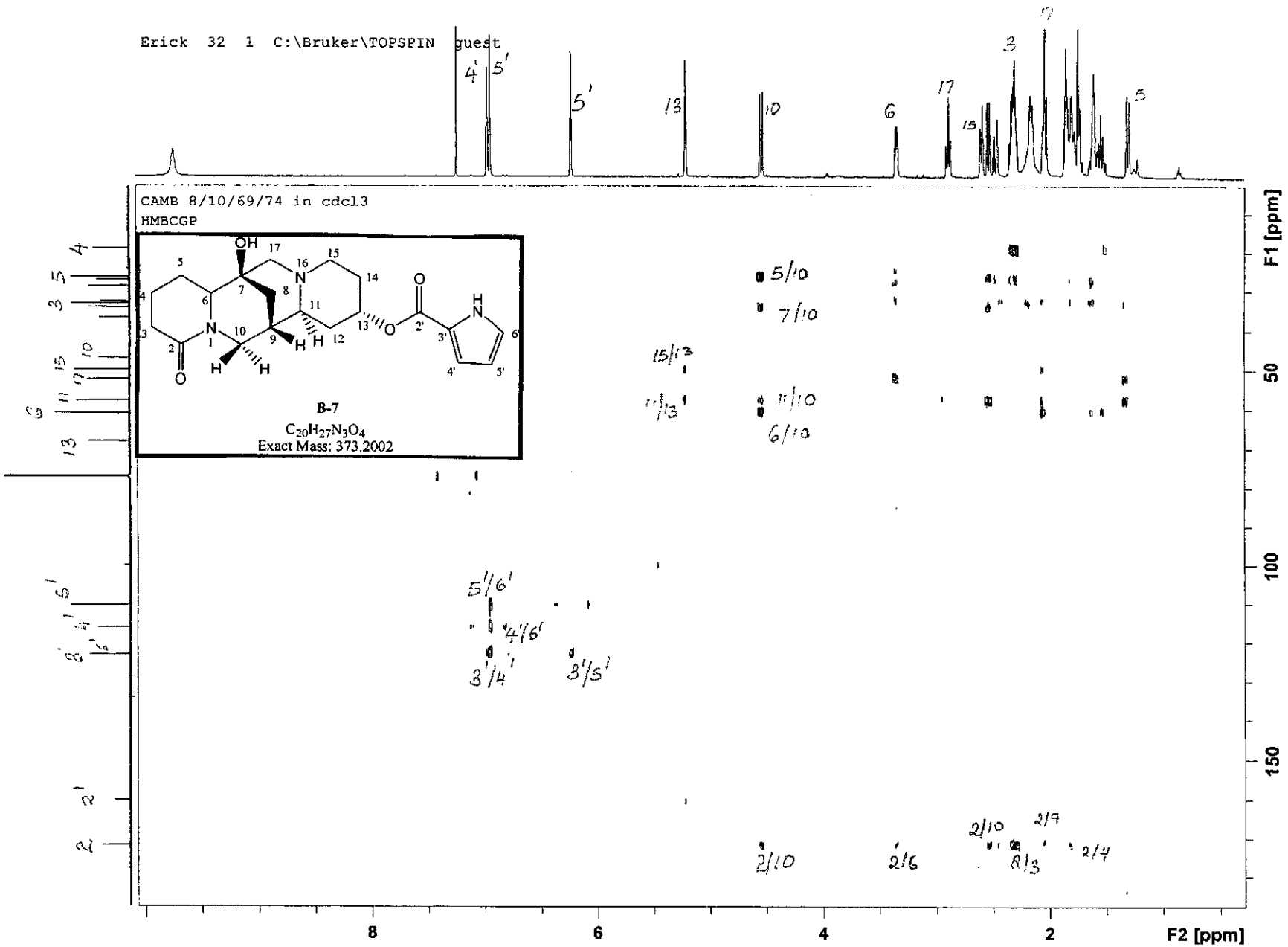
COSY spectrum of calpurnine B7



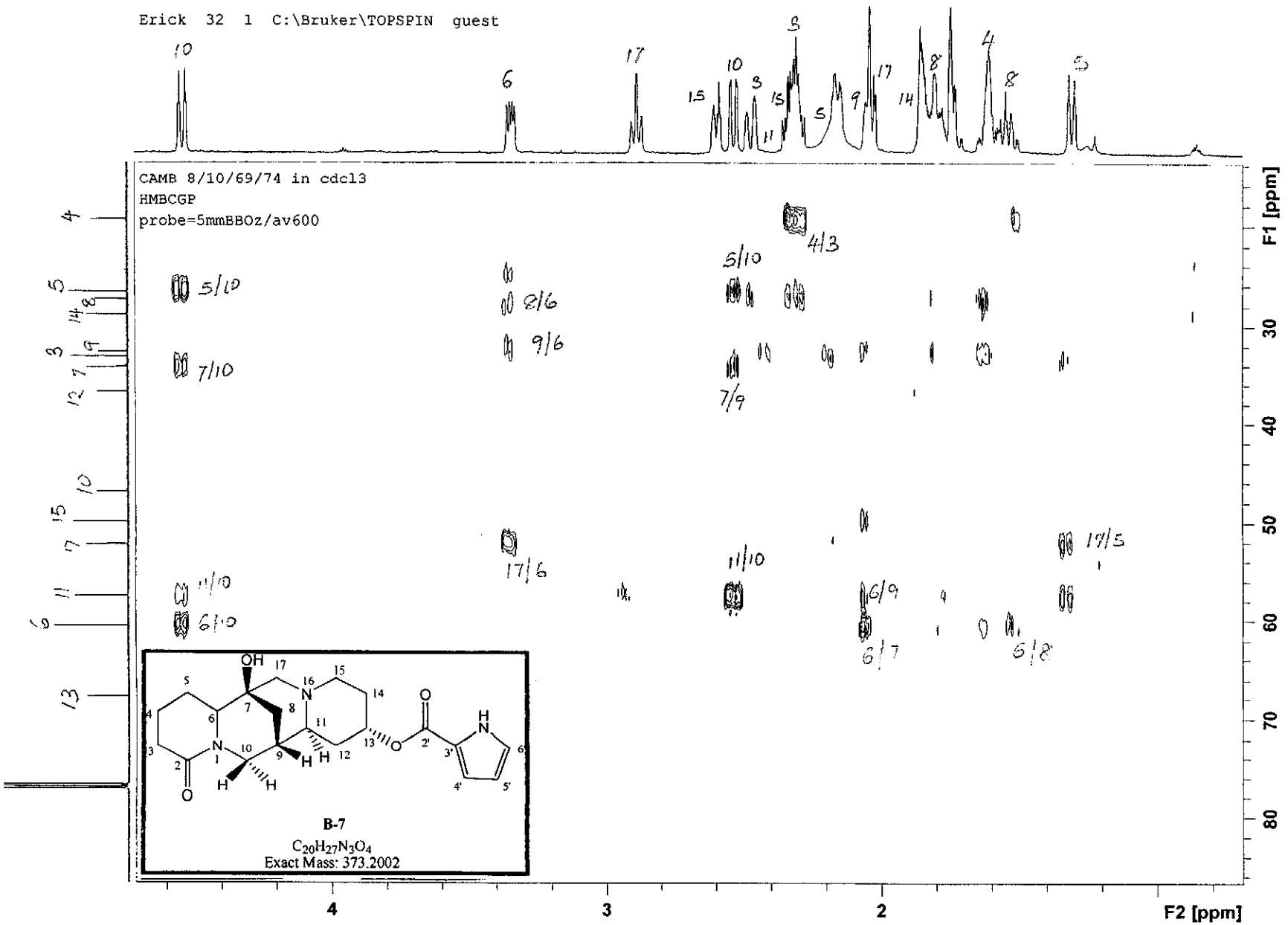
HSQC spectrum of calpurnine B7



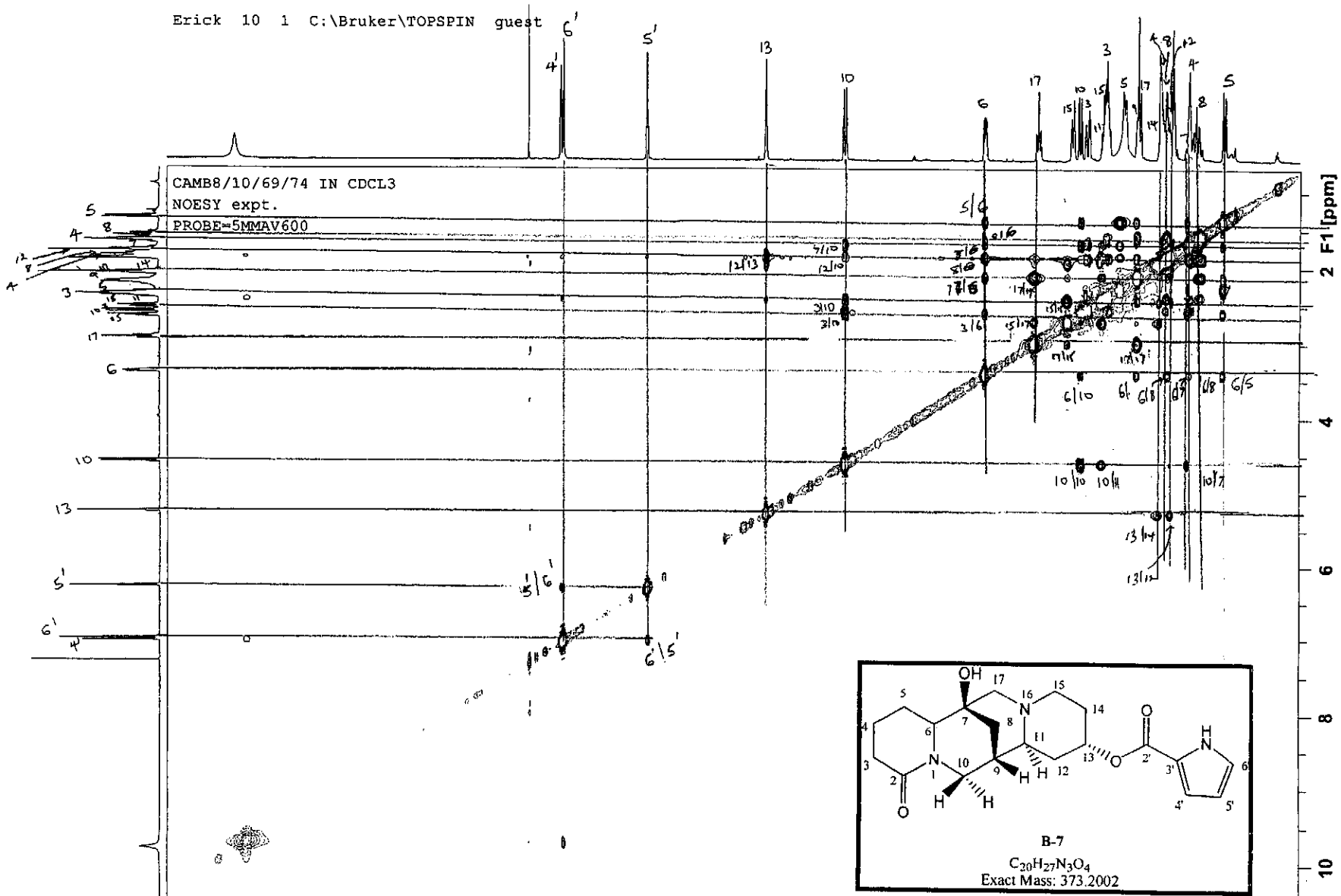
HSQC spectrum of calpurnine B7



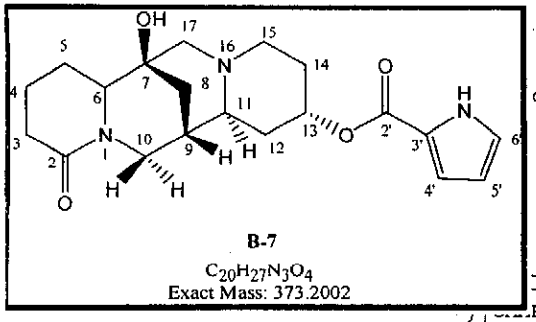
HMBC spectrum of calpurnine B7



HMBC spectrum of calpurnine B7



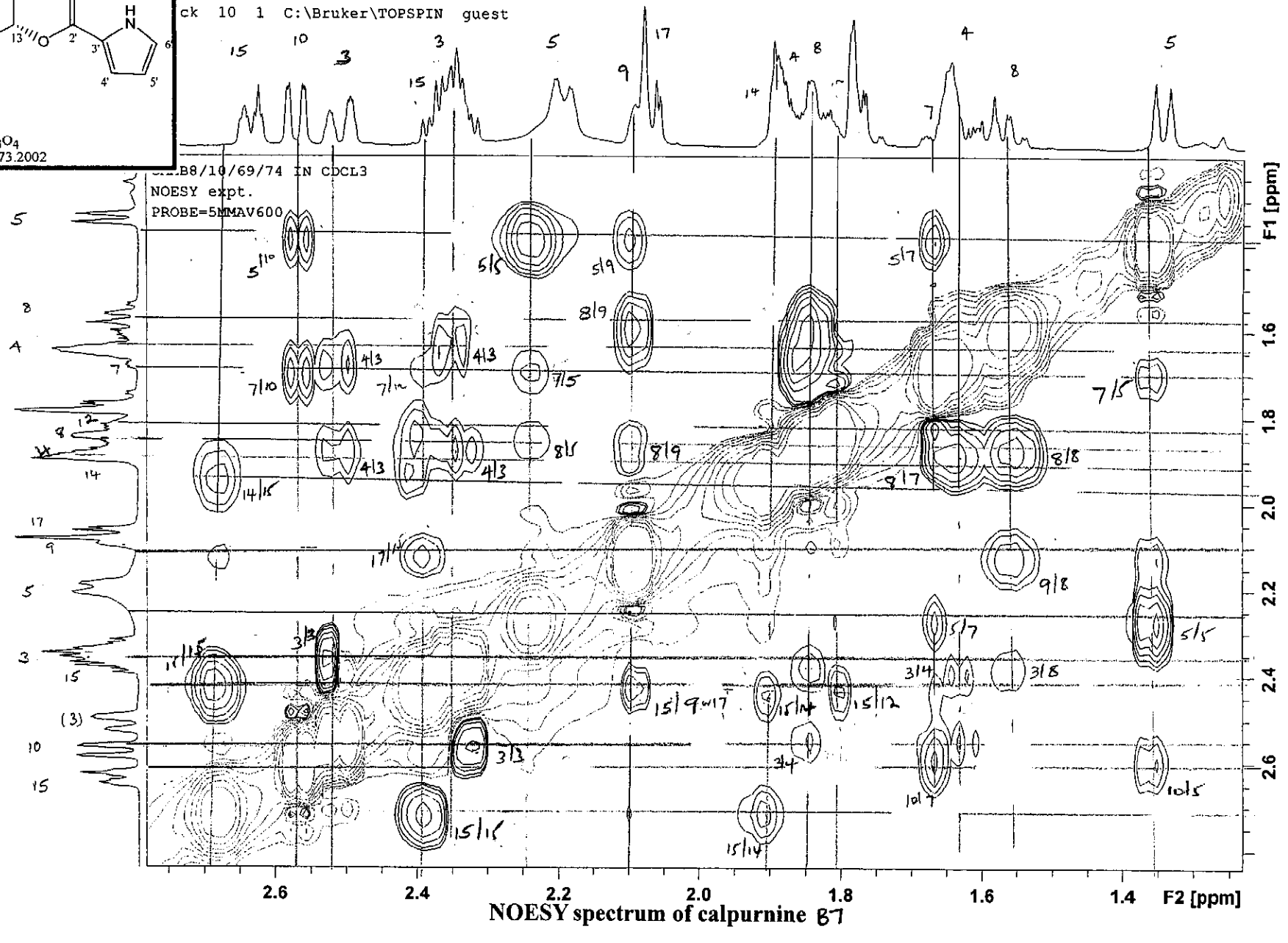
NOESY spectrum of calpurnine B7



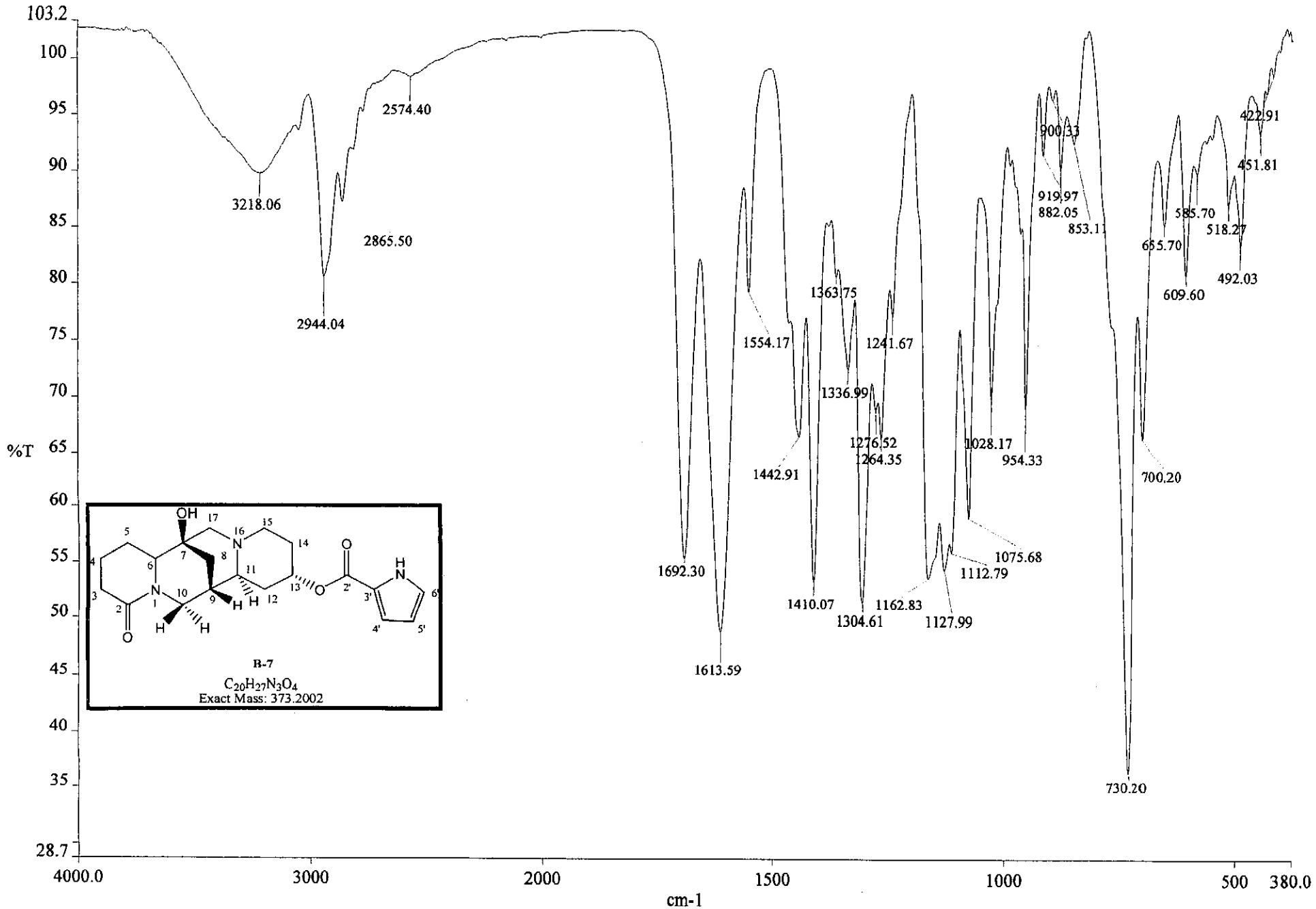
B-7

$C_{20}H_{27}N_3O_4$

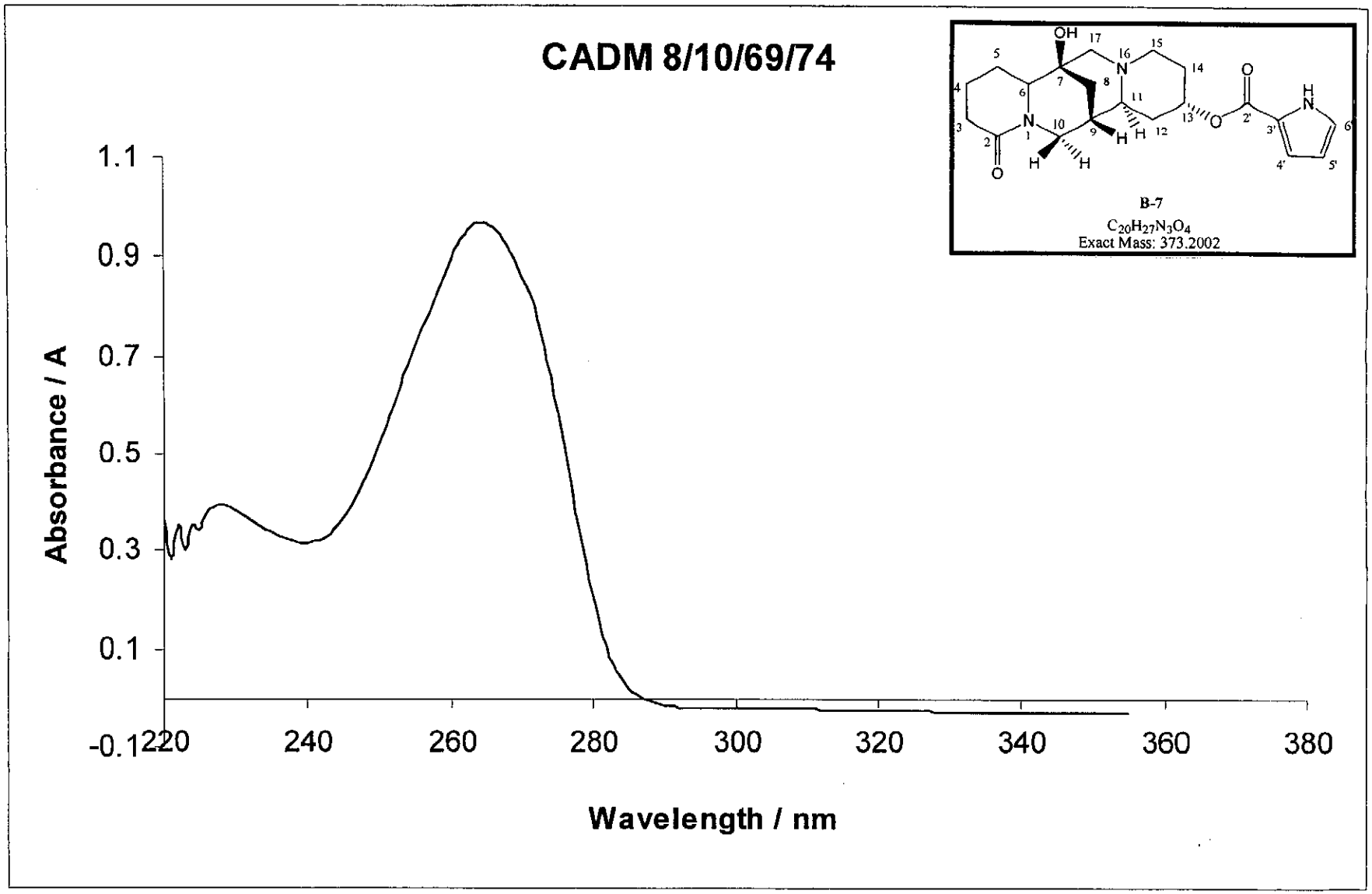
Exact Mass: 373.2002



NOESY spectrum of calpurnine B7



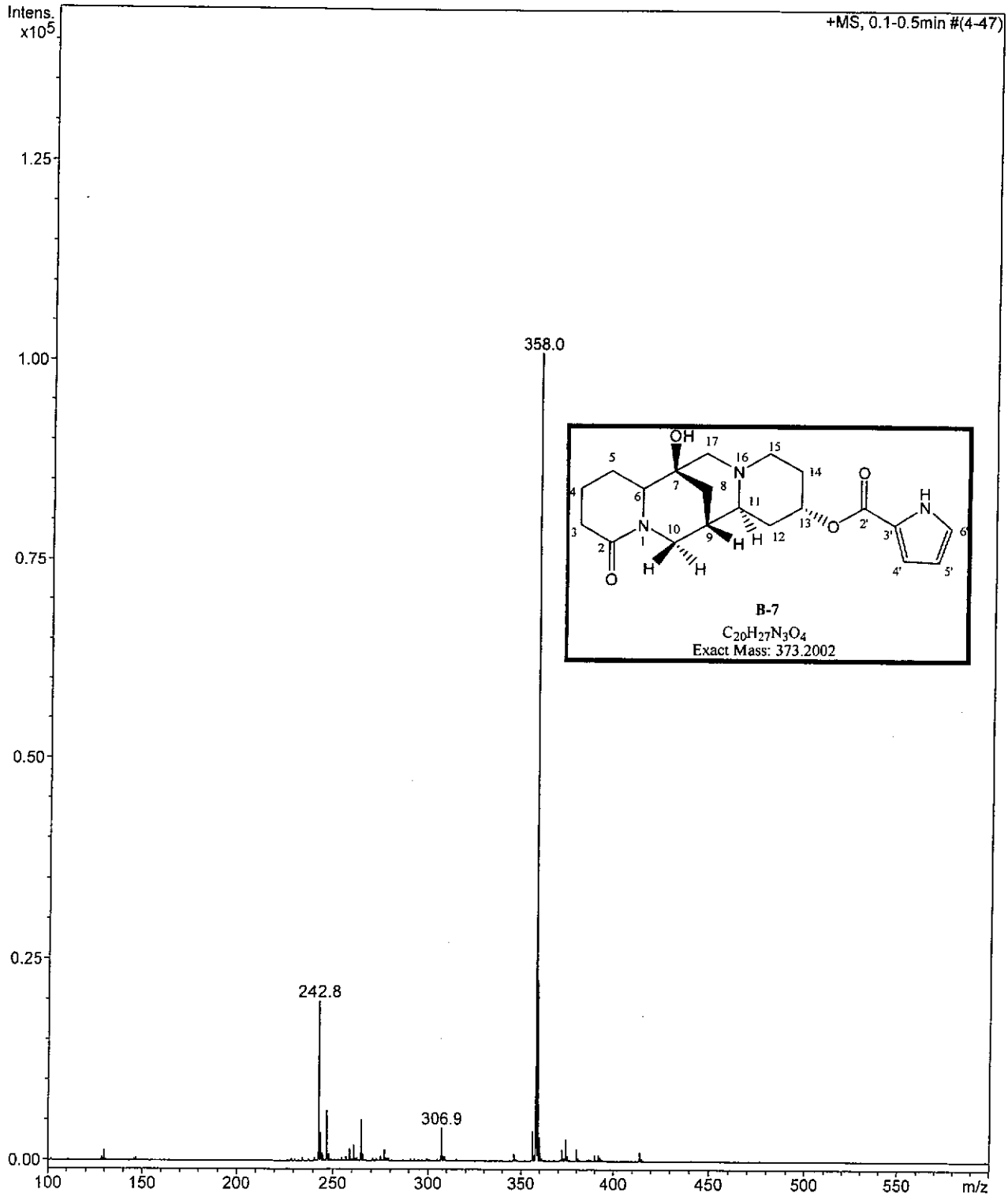
IR spectrum of calpurnine B7



UV spectrum of calpurnine B7

Display Report - All Windows Selected Analysis

Analysis Name: ERICK2.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 10/12/2009 08:31:5
Method: FIA.M **Operator:** Administrator **Acq. Date:** 10/12/2009 7:52:23 P
Sample Name: ERICK2
Analysis Info: SOLUBLE IN METHANOL AND ACN



Mass spectrum of calpurnine B7